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(54) Title: USE OF CONANTOKINS			
(57) Abstract <p>The present invention is directed to the use of conantokin peptides, conantokin peptide derivatives and conantokin peptide chimeras, referred to collectively as conantokins, having 10-30 amino acids, including preferably two or more γ-carboxyglutamic acid residues, for the treatment of neurologic and psychiatric disorders, such as anticonvulsant agents, neuroprotective agents or analgesic agents. Neurologic disorders and psychiatric disorders include epilepsy, convulsions, neurotoxic injury (associated with conditions of hypoxia, anoxia or ischemia which typically follows stroke, cerebrovascular accident, brain or spinal cord trauma, myocardial infarct, physical trauma, drowning, suffocation, perinatal asphyxia, or hypoglycemic events), neurodegeneration (associated with Alzheimer's disease, senile dementia, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, Parkinson's disease, Huntington's disease, Down's Syndrome, Korsakoff's disease, schizophrenia, AIDS dementia, multi-infarct dementia, Binswanger dementia and neuronal damage associated with uncontrolled seizures), chemical toxicity (such as addiction, morphine tolerance, opiate tolerance, opioid tolerance and barbiturate tolerance), pain (acute, chronic, migraine), anxiety, major depression, manic-depressive illness, obsessive-compulsive disorder, schizophrenia and mood disorders (such as bipolar disorder, unipolar depression, dysthymia and seasonal effective disorder) and dystonia (movement disorder), sleep disorder, muscle relaxation and urinary incontinence. In addition, the conantokins are useful for treating HIV infection, ophthalmic indications and memory, learning or cognitive deficits.</p>			

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TITLE OF THE INVENTION

USE OF CONANTOKINS

BACKGROUND OF THE INVENTION

5 The invention relates to the use of relatively short peptides, about 10-30 residues in length, which are naturally available in minute amounts in the venom of the cone snails or analogous to the naturally available peptides, and which include preferably one to two or more γ -carboxyglutamic acid residues for the treatment of neurologic and psychiatric disorders, such as anticonvulsant agents, as neuroprotective agents or for the management of pain.

10 The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference, and for convenience are referenced in the following text by author and date and are listed alphabetically by author in the appended bibliography.

 The predatory cone snails (*Conus*) have developed a unique biological strategy. Their
15 venom contains relatively small peptides that are targeted to various neuromuscular receptors and may be equivalent in their pharmacological diversity to the alkaloids of plants or secondary metabolites of microorganisms. Many of these peptides are among the smallest nucleic acid-encoded translation products having defined conformations, and as such, they are somewhat unusual. Peptides in this size range normally equilibrate among many conformations. Proteins
20 having a fixed conformation are generally much larger.

 The cone snails that produce these peptides are a large genus of venomous gastropods comprising approximately 500 species. All cone snail species are predators that inject venom to capture prey, and the spectrum of animals that the genus as a whole can envenomate is broad. A wide variety of hunting strategies are used, however, every *Conus* species uses fundamentally
25 the same basic pattern of envenomation.

 Several peptides isolated from *Conus* venoms have been characterized. These include the α -, μ - and ω -conotoxins which target nicotinic acetylcholine receptors, muscle sodium channels, and neuronal calcium channels, respectively (Olivera et al., 1985). Conopressins, which are vasopressin analogs, have also been identified (Cruz et al., 1987). In addition,
30 peptides named conantokins have been isolated from *Conus geographus* and *Conus tulipa* (Mena et al., 1990; Haack et al., 1990). These peptides have unusual age-dependent physiological effects: they induce a sleep-like state in mice younger than two weeks and hyperactive behavior in mice older than 3 weeks (Haack et al., 1990).

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The conantokins are structurally unique. In contrast to the well characterized conotoxins from *Conus* venoms, most conantokins do not contain disulfide bonds. However, they contain 4-5 residues of the unusual modified amino acid γ -carboxyglutamic acid. The occurrence of this modified amino acid, which is derived post-translationally from glutamate in a vitamin K-dependent reaction, was unprecedented in a neuropeptide. It has been established that the conantokins have N-methyl-D-aspartate (NMDA) antagonist activity, and consequently target the NMDA receptor. The conantokins reduce glutamate (or NMDA) mediated increases in intracellular Ca^{2+} and cGMP without affecting kainate-mediated events (Chandler et al., 1993). Although these peptides have actions through polyamine responses of the NMDA receptors, the neurochemical profile of these polypeptides is distinct from previously described noncompetitive NMDA antagonists (Skolnick et al., 1992).

The previously identified conantokins are Conantokin G (Con G) and Conantokin T (Con T). Con G has the formula Gly-Glu-Xaa₁-Xaa₁-Leu-Gln-Xaa₂-Asn-Gln-Xaa₂-Leu-Ile-Arg-Xaa₂-Lys-Ser-Asn (SEQ ID NO:1), wherein Xaa₁ and Xaa₂ are γ -carboxyglutamic acid (Gla). The C-terminus preferably contains an amide group. Con T has the formula Gly-Glu-Xaa₁-Xaa₁-Tyr-Gln-Lys-Met-Leu-Xaa₂-Asn-Leu-Arg-Xaa₂-Ala-Glu-Val-Lys-Lys-Asn-Ala (SEQ ID NO:2), wherein Xaa₁ and Xaa₂ are γ -carboxyglutamic acid. The C-terminus preferably contains an amide group. Analogues of Conantokin G have been synthesized and analyzed for their biological activity (Chandler et al., 1993; Zhou et al., 1996). It has been discovered that substitution of the Gla residue at position 4 of Con G destroys its NMDA antagonist properties. Substitution of the Gla residue at position 3 of Con G greatly reduces its NMDA antagonist activity. However, substitutions of the Gla residues at positions 7, 10 and 14 of Con G do not adversely affect potency of the peptide and may even enhance it. (Zhou et al., 1996).

Ischemic damage to the central nervous system (CNS) may result from either global or focal ischemic conditions. Global ischemia occurs under conditions in which blood flow to the entire brain ceases for a period of time, such as may result from cardiac arrest. Focal ischemia occurs under conditions in which a portion of the brain is deprived of its normal blood supply, such as may result from thromboembolytic occlusion of a cerebral vessel, traumatic head or spinal cord injury, edema or brain or spinal cord tumors. Both global and focal ischemic conditions have the potential for widespread neuronal damage, even if the global ischemic condition is transient or the focal condition affects a very limited area.

Epilepsy is a recurrent paroxysmal disorder of cerebral function characterized by sudden brief attacks of altered consciousness, motor activity, sensory phenomena or inappropriate behavior caused by abnormal excessive discharge of cerebral neurons. Convulsive seizures, the most common form of attacks, begin with loss of consciousness and motor control, and tonic or clonic jerking of all extremities but any recurrent seizure pattern may be termed epilepsy. The term primary or idiopathic epilepsy denotes those cases where no cause for the seizures can be identified. Secondary or symptomatic epilepsy designates the disorder when it is associated with such factors as trauma, neoplasm, infection, developmental abnormalities, cerebrovascular disease, or various metabolic conditions. Epileptic seizures are classified as partial seizures (focal, local seizures) or generalized seizures (convulsive or nonconvulsive). Classes of partial seizures include simple partial seizures, complex partial seizures and partial seizures secondarily generalized. Classes of generalized seizures include absence seizures, atypical absence seizures, myoclonic seizures, clonic seizures, tonic seizures, tonic-clonic seizures (*grand mal*) and atonic seizures. Therapeutics having anticonvulsant properties are used in the treatment of seizures. Most therapeutics used to abolish or attenuate seizures act at least through effects that reduce the spread of excitation from seizure foci and prevent detonation and disruption of function of normal aggregates of neurons. Traditional anticonvulsants that have been utilized include phenytoin, phenobarbital, primidone, carbamazepine, ethosuximide, clonazepam and valproate. Several novel and chemically diverse anticonvulsant medications recently have been approved for marketing, including lamotrigine, feribamate, gabapentin and topiramate. For further details of seizures and their therapy, see Rall & Schleifer (1985) and *The Merck Manual* (1992).

It has been shown that neurotransmission mediated through the NMDA receptor complex is associated with seizures (Bowyer, 1982; McNamara et al., 1988), ischemic neuronal injury (Simon et al., 1984; Park et al., 1988) and other phenomena including synaptogenesis (Cline et al., 1987), spatial learning (Morris et al., 1986) and long-term potentiation (Collinridge et al., 1983; Harris et al., 1984; Morris et al., 1986). Regulation of these neuronal mechanisms by NMDA-mediated processes may involve activation of a receptor-gated calcium ion channel (Nowak et al., 1984; Mayer et al., 1987; Ascher and Nowak, 1988).

The NMDA channel is regulated by glycine. This amino acid increases NMDA-evoked currents in various tissues [Johnson and Ascher, 1987; Kleckner and Dingledine, 1988] by increasing the opening frequency of the NMDA channel [Johnson and Ascher, 1987]. Thus,

NMDA-induced calcium influx and intracellular accumulation may be stimulated by glycine [Reynolds et al., 1987; Wroblewski et al., 1989], which interacts with its own distinct site [Williams et al., 1991]. Furthermore, accumulation of intracellular calcium may be implicated in the aforementioned neuropathologies.

5 The NMDA receptor is also involved in a broad spectrum of CNS disorders. For example, during brain ischemia caused by stroke or traumatic injury, excessive amounts of the excitatory amino acid glutamate are released from damaged or oxygen deprived neurons. This excess glutamate binds the NMDA receptor which opens the ligand-gated ion channel thereby allowing Ca^{2+} influx producing a high level of intracellular Ca^{2+} , which activates biochemical
10 cascades resulting in protein, DNA and membrane degradation leading to cell death. This phenomenon, known as excitotoxicity, is also thought to be responsible for the neurological damage associated with other disorders ranging from hypoglycemia and cardiac arrest to epilepsy. In addition, there are reports indicating similar involvement in the chronic neurodegeneration of Huntington's, Parkinson's and Alzheimer's diseases.

15 Parkinson's disease is a progressive, neurodegenerative disorder. The etiology of the disorder is unknown in most cases, but has been hypothesized to involve oxidative stress. The underlying neuropathology in Parkinsonian patients is an extensive degenerations of the pigmented dopamine neurons in the substantia nigra. These neurons normally innervate the caudate and putamen nuclei. Their degeneration results in a marked loss of the neurotransmitter
20 dopamine in the caudate and putamen nuclei. This loss of dopamine and its regulation of neurons in the caudate-putamen leads to the bradykinesia, rigidity, and tremor that are the hallmarks of Parkinson's disease. An animal model has been developed for Parkinson's disease (Zigmond et al., 1987) and has been used to test agents for anti-Parkinsonian activity (Ungerstedt et al., 1973).

25 The dopamine precursor, L-Dopa, is the current therapy of choice in treating the symptoms of Parkinson's disease. However, significant side effects develop with continued use of this drug and with disease progression, making the development of novel therapies important. Recently, antagonists of the NMDA subtype of glutamate receptor have been proposed as potential anti-Parkinsonian agents. (Borman, 1989; Greenamyre and O'Brien, 1991; Olney et
30 al., 1987). In addition, antagonists of NMDA receptors potentiate the behavioral effects of L-Dopa and D1 dopamine receptor stimulation in animal models of Parkinson's disease. (Starr, 1995).

These data suggest that NMDA receptor antagonists may be useful adjuncts to L-Dopa therapy in Parkinson's disease by decreasing the amount of L-Dopa required and thereby reducing undesirable side effects. In addition, antagonists of NMDA receptors have been shown to attenuate free radical mediated neuronal death. Thus, NMDA receptor antagonists may also prevent further degeneration of dopamine neurons in addition to providing symptomatic relief. Finally, NMDA receptor antagonists have been shown to potentiate the contralateral rotations induced by L-Dopa or D1 dopamine receptor antagonists in the animal model.

Pain, and particularly, persistent pain, is a complex phenomenon involving many interacting components. Numerous studies, however, have demonstrated a role for NMDA receptors in mediating persistent pain, and further that NMDA antagonists are effective in animal models of persistent pain. First, administration of NMDA (the agonist) mimics many of the physiological and behavioral effects of painful stimuli (Chapman et al., 1994; Dougherty and Willis, 1991; Coderre and Melzack, 1992; Malmberg and Yaksh, 1992; Bach et al., 1994; Liu et al., 1997). Second, various classes of NMDA antagonists block the "wind up" (progressive augmentation of response caused by repetitive stimulation) of small primary afferent C fibers of the dorsal horn (Davies and Lodge, 1987; Dickenson and Sullivan, 1987; Thompson et al., 1990). Third, release of glutamate and aspartate (agonists at NMDA and non-NMDA glutamatergic receptors) is increased in spinal cord in animal models of persistent pain (Sluka and Westlund, 1992; Malmberg and Yaksh, 1992; Yang et al., 1995). Fourth, NMDA antagonists are effective in animal models of persistent pain (Neugebauer et al., 1993; Coderre, 1993; Coderre and Van Empel, 1994; Yamamoto and Yaksh, 1992; Chaplan et al., 1997; Millan and Seguin, 1994; Rice and McMahon, 1994). Moreover, NMDA antagonists appear to be effective in reducing the tolerance to opioid analgesics seen after chronic administration in animal models of pain (Bilsky et al., 1996; Lufty et al., 1996; Shimoyama et al., 1996; Wong et al., 1996; Elliot et al., 1994; Mao et al., 1994; Dunbar and Yaksh, 1996; Lufty et al., 1995; Trujillo and Akil, 1994; Tiseo et al., 1994; Gutstein and Trujillo, 1993; Kest et al., 1993; Tiseo and Inturrisi, 1993). Finally, severe or prolonged tissue or nerve injury can induce hyperexcitability of dorsal horn neurons of the spinal cord, resulting in persistent pain, an exacerbated response to noxious stimuli (hyperalgesia) and a lowered pain threshold (allodynia). These changes are mediated by NMDA-type glutamate receptors in the spinal cord, whose activation causes release of Substance P, a peptide neurotransmitter made by small-diameter, primary, sensory pain fibres. Injection of

NMDA in the cerebrospinal fluid of the rat spinal cord mimicked the changes that occur with persistent injury and produced pain (Liu et al., 1997).

Neuropsychiatric involvement of the NMDA receptor has also been recognized. Blockage of the NMDA receptor Ca^{2+} channel by the animal anesthetic phencyclidine produces a psychotic state in humans similar to schizophrenia (Johnson et al., 1990). Further, NMDA receptors have also been implicated in certain types of spatial learning (Bliss et al., 1993). In addition, numerous studies have demonstrated a role for NMDA receptors in phenomena associated with addiction to and compulsive use of drugs or ethanol. Furthermore, antagonists of NMDA receptors may be useful for treating addiction-related phenomena such as tolerance, sensitization, physical dependence and craving (for review see, Popik et al., 1995; Spanagel and Zieglansberger, 1997; Trujillo and Akil, 1995).

There are several lines of evidence which suggest that NMDA antagonists may be useful in the treatment of HIV infection. First, the levels of the neurotoxin and NMDA agonist quinolinic acid are elevated in the cerebrospinal fluid of HIV-positive subjects (Heyes et al., 1989) and in murine retrovirus-induced immunodeficiency syndrome (Sei et al., 1996). Second, the envelope glycoprotein of HIV-1 alters NMDA receptor function (Sweetnam et al., 1993). Thirdly, NMDA antagonists can reduce the effects and neurotoxicity of GP-120 (Muller et al., 1996; Raber et al., 1996; Nishida et al., 1996). Fourth, GP-120 and glutamate act synergistically to produce toxicity *in vitro* (Lipton et al., 1991). And finally, memantine, an NMDA antagonist, protects against HIV infection in glial cells *in vitro* (Rytik et al., 1991). For a review of the use of NMDA antagonists in treating HIV infection, see Lipton (1994; 1996).

It is desired to identify additional conantokin peptides and related compounds which target the NMDA receptor. It is further desired to identify compounds which are useful as anticonvulsant, neuroprotective, neuropsychiatric or analgesic agents.

SUMMARY OF THE INVENTION

The present invention is directed to the use of conantokin peptides, conantokin peptide derivatives and conantokin peptide chimeras, referred to collectively as conantokins (unless the context dictates otherwise), having 10-30 amino acids, including preferably one to two or more γ -carboxyglutamic acid residues for the treatment of neurologic or psychiatric disorders, such as

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anticonvulsant agents, as neuroprotective agents or for the management of pain. The conantokins are administered to patients as described further below.

More specifically, the present invention is directed to such uses for conantokin peptides, which include but are not limited to, G, T, R, Sl, Oc, Gm, Ca2, Ca1 and Qu. Conantokin G (Con G) has the formula Gly-Glu-Xaa₁-Xaa₁-Leu-Gln-Xaa₂-Asn-Gln-Xaa₂-Leu-Ile-Arg-Xaa₂-Lys-Ser-Asn (SEQ ID NO:1), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid (Gla). The C-terminus contains a carboxyl or an amide, preferably an amide group. Conantokin T (Con T) has the formula Gly-Glu-Xaa₁-Xaa₁-Tyr-Gln-Lys-Met-Leu-Xaa₂-Asn-Leu-Arg-Xaa₂-Ala-Glu-Val-Lys-Lys-Asn-Ala (SEQ ID NO:2), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or an amide, preferably an amide group. Conatokin L (Con L), has the formula Gly-Glu-Xaa₁-Xaa₁-Val-Ala-Lys-Met-Ala-Ala-Xaa₂-Leu-Ala-Arg-Xaa₂-Asp-Ala-Val-Asn (SEQ ID NO:3), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or an amide, preferably an amide group. Conantokin R (Con R) has the formula: Gly-Glu-Xaa₁-Xaa₁-Val-Ala-Lys-Met-Ala-Ala-Xaa₂-Leu-Ala-Arg-Xaa₂-Asn-Ile-Ala-Lys-Gly-Cys-Lys-Val-Asn-Cys-Tyr-Pro (SEQ ID NO:4), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or an amide, preferably a carboxyl group. The cysteine residues form a disulfide bridge. Conantokin Sl (Con Sl) has the formula: Gly-Asp-Xaa₁-Xaa₁-Tyr-Ser-Lys-Phe-Ile-Xaa₂-Arg-Glu-Arg-Xaa₂-Ala-Gly-Arg-Leu-Asp-Leu-Ser-Lys-Phe-Pro (SEQ ID NO:5), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably a carboxyl group. Conantokin Oc (Con Oc) has the formula: Gly-Glu-Xaa₁-Xaa₁-Tyr-Arg-Lys-Ala-Met-Ala-Xaa₂-Leu-Glu-Ala-Lys-Lys-Ala-Gln-Xaa₂-Ala-Leu-Lys-Ala (SEQ ID NO:6), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably an amide group. Conantokin Gm (Con Gm) has the formula: Gly-Glu-Lys-Xaa₁-Asp-Arg-Asn-Asn-Xaa₂-Ala-Val-Arg-Xaa₂-Arg-Leu-Glu-Glu-Ile (SEQ ID NO:7), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably an amide group. Conantokin Ca2 (Con Ca2) has the formula: Gly-Tyr-Xaa₁-Xaa₁-Asp-Arg-Xaa₂-Ile-Ala-Xaa₂-Tyr-Val-Arg-Xaa₂-Leu-Glu-Glu-Ala (SEQ ID NO:8), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably an amide group. Conantokin Qu (Con Qu) has the formula: Gly-Tyr-Xaa₁-Xaa₁-Asp-Arg-Xaa₂-Val-Ala-Xaa₂-Thr-Val-Arg-Xaa₂-Leu-Asp-Ala-Ala (SEQ ID NO:9),

wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably an amide group. Conantokin Cal (Con Cal) has the formula: Gly-Asn-Asp-Val-Asp-Arg-Lys-Leu-Ala-Xaa₂-Leu-Xaa₂-Xaa₂-Leu-Tyr-Xaa₂-Ile (SEQ ID NO:68), wherein Xaa₂ is preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably an amide group.

The present invention is also directed to such uses for conantokin peptide derivatives. Examples of conantokin peptide derivatives include conantokin peptides in which the γ -carboxyglutamic acid at the Xaa₂ residues in these peptides is replaced by any other amino acids such that their NMDA antagonist activity is not adversely affected. Examples of such replacements include, but are not limited to Ser, Ala, Glu and Tyr. In addition, glutamic acid residues in the peptide can be modified to γ -carboxyglutamate residues. Other derivatives are produced by modification of the amino acids within the conantokin structure. Modified amino acids include those which are described in Roberts et al. (1983). Other derivatives include conantokin peptides in which one or more residues have been deleted.

The present invention is also directed to such uses for conantokin peptide chimeras. Suitable conantokin chimeras are produced by recombination of different segments of two or more conantokin peptides, conantokin peptide derivatives or a peptide encoded by exon 5 of the NMDA receptor, e.g. Lys-Pro-Gly-Arg-Lys (SEQ ID NO:10) or Lys-Pro-Gly-Arg-Lys-Asn (SEQ ID NO:11).

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the time-dependent inhibition of audiogenic seizures by Con R following intracerebroventricular (i.c.v.) administration to Frings audiogenic mice.

Fig. 2 shows the ability of conantokins (Con R (◆), Con T (■) Con G (○)) to block audiogenic seizures in a dose-dependent manner following i.c.v. administration to Frings audiogenic mice.

Fig. 3 shows the dose-dependent reduction in seizure severity following i.c.v. administration to Frings audiogenic mice for conantokins (Con R (◆), Con T (■) Con G (○)).

Fig. 4 shows the dose-dependent blockage of audiogenic sequences by Con R at non-toxic doses. Protection (■) and impairment (○) for Con R are shown.

Fig. 5 shows the dose-response of the anticonvulsant activity of Con G at one (□) and three () minutes following i.c.v. administration to Frings audiogenic mice.

Fig. 6 shows the dose-dependent inhibition of audiogenic seizures following i.c.v. (■) or intravenous (i.v.) (○) administration of conantokin G.

5 Fig. 7 shows the time-dependent inhibition of audiogenic seizures following i.v. administration of conantokin G.

Fig. 8 shows the time-dependent inhibition of audiogenic seizures following per oral (p.o.) administration of conantokin G.

Fig. 9 shows the contralateral rotations following administration of 4 mg/kg L-DOPA (■), 4 mg/kg L-DOPA and 0.5 mM Con G (●) and 4 mg/kg L-DOPA and 5 mM Con G (▲) in a
10 Parkinson's disease animal model.

Fig. 10 shows the ipsilateral rotations following administration of 4 mg/kg L-DOPA (■), 4 mg/kg L-DOPA and 0.5 mM Con G (●) and 4 mg/kg L-DOPA and 5 mM Con G (▲) in a Parkinson's disease animal model.

15 Fig. 11 shows the number of grooming episodes following administration of SKF 38393 (■) or the combination of SKF 38393 and Con G (●).

Fig. 12 shows the number of net contralateral turns following administration of SKF 38393 (■) or the combination of SKF 38393 and Con G (●). The asterisks indicate statistical significance at $p < 0.05$.

20 Fig. 13 shows the number of rearing episodes following administration of SKF 38393 (■) or the combination of SKF 38393 and Con G (●).

Fig. 14 shows the number of cage lengths crossed following administration of SKF 38393 (■) or the combination of SKF 38393 and Con G (●). Statistical significance is shown at $p < 0.05$ (*), $p < 0.01$ (†) or $p < 0.01$ (§).

25 Fig. 15 shows the number of contralateral turns following administration of SKF 38393 (■) or the combination of SKF 38393 and Con G (●). The asterisks indicate statistical significance at $p < 0.05$.

Fig. 16 shows the number of contralateral turns following administration of SKF 38393 (■) or the combination of SKF 38393 and Con G (●).

30 Fig. 17 shows the effect of Con G (▲) and Con T (▽) on bladder contraction amplitude.

Fig. 18 shows the effect of Con G (▲) and Con T (▽) on EUS EM G activity.

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Fig. 19 shows the peptide stability of ECon G (■), Con G (▲), Con R (▼) and Con T (◆) in 20% Frings mouse serum.

Fig. 20 shows the peptide stability of ECon G (■), Con G (▲), Con R (●) and Con T (□) in 20% Frings mouse liver homogenate.

5 Fig. 21 shows the stability of conantokin G in physiological saline at pH 6.0 at 37°C (■) and (▲).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention is directed to the use of conantokin peptides, conantokin peptide
10 derivatives and conantokin peptide chimeras, referred to collectively as conantokins (unless the context dictates otherwise), having 10-30 amino acids, including preferably one to two or more γ -carboxyglutamic acid residues, for the treatment of neurologic and psychiatric disorders, such as anticonvulsant agents, as neuroprotective agents or for the management of pain, e.g. as analgesic agents. Neurologic disorders and psychiatric disorders as used herein are intended to
15 include such disorders as grouped together in *The Merck Manual of Diagnosis and Therapy*, inclusive of the disorders discussed herein.

More specifically, the present invention is directed to the use of conantokins for the treatment and alleviation of epilepsy and as a general anticonvulsant agent. The present invention is also directed to the use of conantokins for reducing neurotoxic injury associated
20 with conditions of hypoxia, anoxia or ischemia which typically follows stroke, cerebrovascular accident, brain or spinal cord trauma, myocardial infarct, physical trauma, drowning, suffocation, perinatal asphyxia, or hypoglycemic events. The present invention is further directed to the use of conantokins for treating neurodegeneration associated with Alzheimer's disease, senile dementia, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, Parkinson's disease,
25 Huntington's disease, Down's Syndrome, Korsakoff's disease, schizophrenia, AIDS dementia, multi-infarct dementia, Binswanger dementia and neuronal damage associated with uncontrolled seizures. The present invention is also directed to the use of conantokins for treating chemical toxicity, such as addiction, drug craving, alcohol abuse, morphine tolerance, opioid tolerance and barbiturate tolerance. The present invention is further directed to treating psychiatric disorders,
30 such as anxiety, major depression, manic-depressive illness, obsessive-compulsive disorder, schizophrenia and mood disorders (such as bipolar disorder, unipolar depression, dysthymia and

seasonal effective disorder). The conantokins are also useful for treating ophthalmic disorders. The present invention is also directed to treating additional neurological disorders, such as dystonia (movement disorder), sleep disorder, muscle relaxation and urinary incontinence. In addition, the conantokins are useful for memory/cognition enhancement, i.e., treating memory, learning or cognitive deficits. The present invention is also useful in the treatment of HIV infection. Finally, the present invention is directed to the use of conantokins for controlling pain, e.g. as analgesic agents, and the treatment of migraine, acute pain or persistent pain. They can be used prophylactically and also to relieve the symptoms associated with a migraine episode.

- 10 The present invention is directed to the uses described herein for conantokin peptides, which include but are not limited to G, T, R, Sl, Oc, L, Gm, Ca2, Ca1 and Qu. Conantokin G (Con G) has the formula Gly-Glu-Xaa₁-Xaa₁-Leu-Gln-Xaa₂-Asn-Gln-Xaa₂-Leu-Ile-Arg-Xaa₂-Lys-Ser-Asn (SEQ ID NO:1), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid (Gla). The C-terminus contains a carboxyl or an amide, preferably an amide group.
- 15 Conantokin T (Con T) has the formula Gly-Glu-Xaa₁-Xaa₁-Tyr-Gln-Lys-Met-Leu-Xaa₂-Asn-Leu-Arg-Xaa₂-Ala-Glu-Val-Lys-Lys-Asn-Ala (SEQ ID NO:2), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or an amide, preferably an amide group.
- 20 Conantokin L (Con L), has the formula Gly-Glu-Xaa₁-Xaa₁-Val-Ala-Lys-Met-Ala-Ala-Xaa₂-Leu-Ala-Arg-Xaa₂-Asp-Ala-Val-Asn (SEQ ID NO:3), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or an amide, preferably an amide group.
- 25 Conantokin R (Con R) has the formula: Gly-Glu-Xaa₁-Xaa₁-Val-Ala-Lys-Met-Ala-Ala-Xaa₂-Leu-Ala-Arg-Xaa₂-Asn-Ile-Ala-Lys-Gly-Cys-Lys-Val-Asn-Cys-Tyr-Pro (SEQ ID NO:4), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or an amide, preferably a carboxyl group. The cysteine residues form a disulfide bridge.
- 30 Conantokin Sl (Con Sl) has the formula: Gly-Asp-Xaa₁-Xaa₁-Tyr-Ser-Lys-Phe-Ile-Xaa₂-Arg-Glu-Arg-Xaa₂-Ala-Gly-Arg-Leu-Asp-Leu-Ser-Lys-Phe-Pro (SEQ ID NO:5), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably a carboxyl group.
- Conantokin Oc (Con Oc) has the formula: Gly-Glu-Xaa₁-Xaa₁-Tyr-Arg-Lys-Ala-Met-Ala-Xaa₂-Leu-Glu-Ala-Lys-Lys-Ala-Gln-Xaa₂-Ala-Leu-Lys-Ala (SEQ ID NO:6), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably an amide group.
- Conantokin Gm (Con Gm) has the

formula: Gly-Glu-Lys-Xaa₁-Asp-Arg-Asn-Asn-Xaa₂-Ala-Val-Arg-Xaa₂-Arg-Leu-Glu-Glu-Ile (SEQ ID NO:7), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably an amide group. Conantokin Ca2 (Con Ca2) has the formula: Gly-Tyr-Xaa₁-Xaa₁-Asp-Arg-Xaa₂-Ile-Ala-Xaa₂-Tyr-Val-Arg-Xaa₂-Leu-Glu-Glu-Ala (SEQ ID NO:8), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably an amide group. Conantokin Qu (Con Qu) has the formula: Gly-Tyr-Xaa₁-Xaa₁-Asp-Arg-Xaa₂-Val-Ala-Xaa₂-Thr-Val-Arg-Xaa₂-Leu-Asp-Ala-Ala (SEQ ID NO:9), wherein Xaa₁ and Xaa₂ are preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably an amide group. Conantokin Cal (Con Cal) has the formula: Gly-Asn-Asp-Val-Asp-Arg-Lys-Leu-Ala-Xaa₂-Leu-Xaa₂-Xaa₂-Leu-Tyr-Xaa₂-Ile (SEQ ID NO:68), wherein Xaa₂ is preferably γ -carboxyglutamic acid. The C-terminus contains a carboxyl or amide, preferably an amide group.

The present invention is further directed to the uses described herein of conantokin peptide derivatives. Examples of suitable derivatives include, but are not limited to those described herein. In one embodiment, the γ -carboxyglutamic acid at the Xaa₂ residues in the above peptides may be replaced by any other amino acids without adversely affecting their NMDA antagonist activity. Examples of such amino acid replacements include, but are not limited to, Ser, Ala, Glu and Tyr. In addition, glutamic acid residues in the peptide can be modified to γ -carboxyglutamate residues. Substitutions of one amino acid for another can be made at one or more additional sites within the above conantokin peptides, and may be made to modulate one or more of the properties of the peptides. Substitutions of this kind are preferably conservative, i.e., one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example: alanine to serine, arginine to lysine, asparagine to glutamine or histidine, glycine to proline, leucine to valine or isoleucine, serine to threonine, phenylalanine to tyrosine, and the like. Other derivatives are produced by modification of the amino acids within the conantokin structure. Modified amino acids include those which are described in Roberts et al. (1983). Other derivatives include conantokin peptides in which one or more residues have been deleted. For example, one such derivative is conantokin G in which the five C-terminal amino acids have been deleted. The activity of such derivatives can easily be determined in assays known in the art, including but not limited to the assays disclosed herein.

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Finally, the present invention is directed to the uses described herein of conantokin peptide chimeras. Suitable conantokin peptide chimeras are produced by recombination of different segments of two or more conantokin peptides, conantokin peptide derivatives or the peptide encoded by exon 5 of the NMDA receptor, e.g. Lys-Pro-Gly-Arg-Lys (SEQ ID NO:10) or Lys-Pro-Gly-Arg-Lys-Asn (SEQ ID NO:11). The conantokin peptides and conantokin peptide derivatives can be divided into, for example, four domains as shown in Table 1. Table 1 is not meant to be exclusive and domains of conantokin peptides or conantokin peptide derivatives not set forth in Table 1 can also be easily identified. The SEQ ID NOs are in parentheses. "γ" is γ-carboxyglutamic acid.

TABLE 1

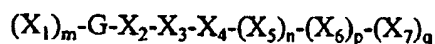
Domains of Conantokin Peptides and Derivatives

Conantokin	I	II	III	IV
Con G	GE $\gamma\gamma$ (12)	LQ γ NQ γ (13)	LIR γ (14)	KSN
Con T	GE $\gamma\gamma$ (12)	YQKML γ (15)	NLR γ (16)	AEVKKNA (17)
Con R	GE $\gamma\gamma$ (12)	VAKMAA γ (18)	LAR γ (19)	NIAGCKVNCYP (20)
Con L	GE $\gamma\gamma$ (12)	VAKMAA γ (18)	LAR γ (19)	DAVN (21)
A ^{7,10,14} Con G	GE $\gamma\gamma$ (12)	LQANQA (22)	LIRA (23)	KSN
A ⁷ Con G	GE $\gamma\gamma$ (12)	LQANQ γ (24)	LIR γ (14)	KSN
S ⁷ Con G	GE $\gamma\gamma$ (12)	LQSNQ γ (25)	LIR γ (14)	KSN
T ⁷ Con G	GE $\gamma\gamma$ (12)	LQTNQ γ (26)	LIR γ (14)	KSN
Con SI	GD $\gamma\gamma$ (27)	YSKFI γ (28)	RER γ (29)	AGRLDLSKFP (30)
Con Oc	GE $\gamma\gamma$ (12)	YRKAMA γ (31)	LEAKKAQ γ (32)	ALKA (33)
Con Qu	GY $\gamma\gamma$ (34)	DR γ VA γ (35)	TVR γ (36)	LDAA (37)
Con Ca2	GY $\gamma\gamma$ (12)	DR γ IA γ (38)	TVR γ (36)	LEEA (39)
Con Gm	GAK γ (40)	DRNNA γ (41)	AVR γ (42)	RLEEI (43)
Con Ca1	GNDV (69)	DRKLA γ (70)	LE γ (71)	LYEI (72)

The conantokin peptide chimeras are prepared by combining any one of the individual elements of each domain with any one of the elements of the other domains. Thus, conatokin peptide chimeras are prepared by combining (a) any one of domain I, (b) any one of domain II, (c) any one of domain III and (d) any one of domain IV. Additional conantokin peptide chimeras are prepared by combining domain I to the C terminal end of a peptide encoded by exon 5 of the NMDA receptor. Examples of the latter peptide include Lys-Pro-Gly-Arg-Lys (SEQ ID NO:10) and Lys-Pro-Gly-Arg-Lys-Asn (SEQ ID NO:11). The activity of such

chimeras can easily be determined in assays known in the art, including but not limited to the assays disclosed herein.

In view of the definitions for conantokin peptides, conantokin peptide derivatives and conantokin peptide chimeras, a generic formula for conantokins useful in the present invention is derived. This generic formula is as follows:



wherein

X_1 is Lys-Pro-Gly-Arg-Lys (SEQ ID NO:10) or Lys-Pro-Gly-Arg-Lys-Asn (SEQ ID NO:11),

10 X_2 is any amino acid,

X_3 is any amino acid,

X_4 is any amino acid,

X_5 is a peptide having 1-7 amino acid residues,

X_6 is a peptide having 1-4 amino acid residues,

15 X_7 is a peptide having 1-12 amino acid residues,

m , n , p , and q are independently 0 or 1, with the proviso that when m is 1, then n , p and q are each 0.

It is preferred that X_2 is Glu, Asp, Tyr, Ala or Asn, X_3 is Lys, Glu, Gla, Asp, Tyr, Ala, Ser or phosphoserine, X_4 is Glu, Gla, Asp, Ala, Ser or phosphoserine and n is 1. More preferably, X_4 is Gla, and most preferably, X_3 and X_4 are each Gla.

The conantokin peptides, conantokin peptide derivatives, conantokin peptide chimeras and conantokins of the generic formula above, collectively referred to as conantokins, have anticonvulsant activity in Frings audiogenic seizure susceptible mice and in syndrome-specific seizure animal models. These conantokins also have activity in animal pain models. These conantokins further have activity in *in vitro* assays for protection from neurotoxicity. These conantokins also have activity in animal models for Parkinson's disease. Thus, the conantokins of the present invention are useful as anticonvulsant agents, as neuroprotective agents, as analgesic agents, for managing pain and for treating neurodegenerative disorders. The conantokins of the present inventions are particularly useful as such agents for treating neurologic disorders and psychiatric disorders that result from an overstimulation of excitatory amino acid receptors. That is, the invention pertains to disorders in which the pathophysiology

involves excessive excitation of nerve cells by excitatory amino acids or agonists of the NMDA receptor(s). The conantokins are administered to patients as described further below.

These peptides, derivatives and chimeras are sufficiently small to be chemically synthesized. General chemical syntheses for preparing the foregoing conantokin peptides, conantokin peptide
5 derivatives and conantokin peptide chimeras are described hereinafter, along with specific chemical synthesis of one conantokin peptide and indications of biological activities of these synthetic products. Various ones of the conantokin peptides can also be obtained by isolation and purification from specific *Conus* species using the technique described in U.S. Patent No. 4,447,356 (Olivera et al., 1984), the disclosure of which is incorporated herein by reference.

10 Although the conantokin peptides of the present invention can be obtained by purification from cone snails, because the amounts of conantokin peptides obtainable from individual snails are very small, the desired substantially pure conantokin peptides are best practically obtained in commercially valuable amounts by chemical synthesis using solid-phase strategy. For example, the yield from a single cone snail may be about 10 micrograms or less of conantokin peptide. By
15 "substantially pure" is meant that the peptide is present in the substantial absence of other biological molecules of the same type; it is preferably present in an amount of at least about 85% purity and preferably at least about 95% purity. Chemical synthesis of biologically active conantokin peptides depends of course upon correct determination of the amino acid sequence.

The conantokin peptides can also be produced by recombinant DNA techniques well
20 known in the art. Such techniques are described by Sambrook et al. (1979). The peptides produced in this manner are isolated, reduced if necessary, and oxidized to form the correct disulfide bonds.

One method of forming disulfide bonds in the conantokin peptides of the present invention is the air oxidation of the linear peptides for prolonged periods under cold room
25 temperatures or at room temperature. This procedure results in the creation of a substantial amount of the bioactive, disulfide-linked peptides. The oxidized peptides are fractionated using reverse-phase high performance liquid chromatography (HPLC) or the like, to separate peptides having different linked configurations. Thereafter, either by comparing these fractions with the elution of the native material or by using a simple assay, the particular fraction having the
30 correct linkage for maximum biological potency is easily determined. However, because of the

dilution resulting from the presence of other fractions of less biopotency, a somewhat higher dosage may be required.

The peptides are synthesized by a suitable method, such as by exclusively solid-phase techniques, by partial solid-phase techniques, by fragment condensation or by classical solution
5 couplings.

In conventional solution phase peptide synthesis, the peptide chain can be prepared by a series of coupling reactions in which constituent amino acids are added to the growing peptide chain in the desired sequence. Use of various coupling reagents, e.g., dicyclohexylcarbodiimide or diisopropylcarbonyldimidazole, various active esters, e.g., esters of N-hydroxyphthalimide or N-
10 hydroxy-succinimide, and the various cleavage reagents, to carry out reaction in solution, with subsequent isolation and purification of intermediates, is well known classical peptide methodology. Classical solution synthesis is described in detail in the treatise, "Methoden der Organischen Chemie (Houben-Weyl): Synthese von Peptiden," (1974). Techniques of exclusively solid-phase synthesis are set forth in the textbook, "Solid-Phase Peptide Synthesis,"
15 (Stewart and Young, 1969), and are exemplified by the disclosure of U.S. Patent 4,105,603 (Vale et al., 1978). The fragment condensation method of synthesis is exemplified in U.S. Patent 3,972,859 (1976). Other available syntheses are exemplified by U.S. Patents No. 3,842,067 (1974) and 3,862,925 (1975). The synthesis of peptides containing γ -carboxyglutamic acid residues is exemplified by Rivier et al. (1987), Nishiuchi et al. (1993) and Zhou et al. (1996).

Common to such chemical syntheses is the protection of the labile side chain groups of the various amino acid moieties with suitable protecting groups which will prevent a chemical reaction from occurring at that site until the group is ultimately removed. Usually also common is the protection of an α -amino group on an amino acid or a fragment while that entity reacts at the carboxyl group, followed by the selective removal of the α -amino protecting group to allow
25 subsequent reaction to take place at that location. Accordingly, it is common that, as a step in such a synthesis, an intermediate compound is produced which includes each of the amino acid residues located in its desired sequence in the peptide chain with appropriate side-chain protecting groups linked to various ones of the residues having labile side chains.

As far as the selection of a side chain amino protecting group is concerned, generally one
30 is chosen which is not removed during deprotection of the α -amino groups during the synthesis. However, for some amino acids, e.g., His, protection is not generally necessary. In selecting a

particular side chain protecting group to be used in the synthesis of the peptides, the following general rules are followed: (a) the protecting group preferably retains its protecting properties and is not split off under coupling conditions, (b) the protecting group should be stable under the reaction conditions selected for removing the α -amino protecting group at each step of the synthesis, and (c) the side chain protecting group must be removable, upon the completion of the synthesis containing the desired amino acid sequence, under reaction conditions that will not undesirably alter the peptide chain.

It should be possible to prepare many, or even all, of these peptides using recombinant DNA technology. However, when peptides are not so prepared, they are preferably prepared using the Merrifield solid-phase synthesis, although other equivalent chemical syntheses known in the art can also be used as previously mentioned. Solid-phase synthesis is commenced from the C-terminus of the peptide by coupling a protected α -amino acid to a suitable resin. Such a starting material can be prepared by attaching an α -amino-protected amino acid by an ester linkage to a chloromethylated resin or a hydroxymethyl resin, or by an amide bond to a benzhydrylamine (BHA) resin or paramethylbenzhydrylamine (MBHA) resin. Preparation of the hydroxymethyl resin is described by Bodansky et al. (1966). Chloromethylated resins are commercially available from Bio Rad Laboratories (Richmond, CA) and from Lab. Systems, Inc. The preparation of such a resin is described by Stewart et al. (1969). BHA and MBHA resin supports are commercially available, and are generally used when the desired polypeptide being synthesized has an unsubstituted amide at the C-terminus. Thus, solid resin supports may be any of those known in the art, such as one having the formulae $-O-CH_2$ -resin support, $-NH$ BHA resin support, or $-NH$ -MBHA resin support. When the unsubstituted amide is desired, use of a BHA or MBHA resin is preferred, because cleavage directly gives the amide. In case the N-methyl amide is desired, it can be generated from an N-methyl BHA resin. Should other substituted amides be desired, the teaching of U.S. Patent No. 4,569,967 (Kornreich et al., 1986) can be used, or should still other groups than the free acid be desired at the C-terminus, it may be preferable to synthesize the peptide using classical methods as set forth in the Houben-Weyl text (1974).

The C-terminal amino acid, protected by Boc or Fmoc and by a side-chain protecting group, if appropriate, can be first coupled to a chloromethylated resin according to the procedure set forth in K. Horiki et al. (1978), using KF in DMF at about 60°C for 24 hours with stirring,

when a peptide having free acid at the C-terminus is to be synthesized. Following the coupling of the BOC-protected amino acid to the resin support, the α -amino protecting group is removed, as by using trifluoroacetic acid (TFA) in methylene chloride or TFA alone. The deprotection is carried out at a temperature between about 0°C and room temperature. Other standard cleaving reagents, such as HCl in dioxane, and conditions for removal of specific α -amino protecting groups may be used as described in Schroder & Lubke (1965).

After removal of the α -amino-protecting group, the remaining α -amino- and side chain-protected amino acids are coupled step-wise in the desired order to obtain the intermediate compound defined hereinbefore, or as an alternative to adding each amino acid separately in the synthesis, some of them may be coupled to one another prior to addition to the solid phase reactor. Selection of an appropriate coupling reagent is within the skill of the art. Particularly suitable as a coupling reagent is N,N'-dicyclohexylcarbodiimide (DCC, DIC, HBTU, HATU, TBTU in the presence of HoBt or HoAt).

The activating reagents used in the solid phase synthesis of the peptides are well known in the peptide art. Examples of suitable activating reagents are carbodiimides, such as N,N'-diisopropylcarbodiimide and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide. Other activating reagents and their use in peptide coupling are described by Schroder & Lubke (1965) and Kapoor (1970).

Each protected amino acid or amino acid sequence is introduced into the solid-phase reactor in about a twofold or more excess, and the coupling may be carried out in a medium of dimethylformamide (DMF):CH₂Cl₂ (1:1) or in DMF or CH₂Cl₂ alone. In cases where intermediate coupling occurs, the coupling procedure is repeated before removal of the α -amino protecting group prior to the coupling of the next amino acid. The success of the coupling reaction at each stage of the synthesis, if performed manually, is preferably monitored by the ninhydrin reaction, as described by Kaiser et al. (1970). Coupling reactions can be performed automatically, as on a Beckman 990 automatic synthesizer, using a program such as that reported in Rivier et al. (1978).

After the desired amino acid sequence has been completed, the intermediate peptide can be removed from the resin support by treatment with a reagent, such as liquid hydrogen fluoride or TFA (if using Fmoc chemistry), which not only cleaves the peptide from the resin but also cleaves all remaining side chain protecting groups and also the α -amino protecting group at the

N-terminus if it was not previously removed to obtain the peptide in the form of the free acid. If Met is present in the sequence, the Boc protecting group is preferably first removed using trifluoroacetic acid (TFA)/ethanedithiol prior to cleaving the peptide from the resin with HF to eliminate potential S-alkylation. When using hydrogen fluoride or TFA for cleaving, one or
5 more scavengers such as anisole, cresol, dimethyl sulfide and methylethyl sulfide are included in the reaction vessel.

Cyclization of the linear peptide is preferably affected, as opposed to cyclizing the peptide while a part of the peptido-resin, to create bonds between Cys residues. To effect such a disulfide cyclizing linkage, fully protected peptide can be cleaved from a hydroxymethylated
10 resin or a chloromethylated resin support by ammonolysis, as is well known in the art, to yield the fully protected amide intermediate, which is thereafter suitably cyclized and deprotected. Alternatively, deprotection, as well as cleavage of the peptide from the above resins or a benzhydrylamine (BHA) resin or a methylbenzhydrylamine (MBHA), can take place at 0°C with hydrofluoric acid (HF) or TFA, followed by oxidation as described above.

15 The peptides are also synthesized using an automatic synthesizer. Amino acids are sequentially coupled to an MBHA Rink resin (typically 100 mg of resin) beginning at the C-terminus using an Advanced Chemtech 357 Automatic Peptide Synthesizer. Couplings are carried out using 1,3-diisopropylcarbodiimide in N-methylpyrrolidinone (NMP) or by 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and diethylisopro-
20 pylamine (DIEA). The Fmoc protecting group is removed by treatment with a 20% solution of piperidine in dimethylformamide (DMF). Resins are subsequently washed with DMF (twice), followed by methanol and NMP.

The conantokins are antagonists of the NMDA receptor subunits and are useful as anticonvulsant agents, as neuroprotective agents, as analgesic agents, for managing pain and for
25 treating neurodegenerative disorders. The conantokins of the present inventions are particularly useful as such agents for treating neurologic disorders and psychiatric disorders that result from an overstimulation of excitatory amino acid receptors. That is, the invention pertains particularly to disorders in which the pathophysiology involves excessive excitation of nerve cells by excitatory amino acids or agonists of the NMDA receptor(s). Thus, the conantokins of
30 the present invention are useful for the treatment and alleviation of epilepsy and as a general anticonvulsant agent. The use of conantokins in these conditions includes the administration of

a conantokin in a therapeutically effective amount to patients in need of treatment. The conantokins can be used to treat the seizures, to reduce their effects and to prevent seizures.

The conantokins are also useful to reduce neurotoxic injury associated with conditions of hypoxia, anoxia or ischemia which typically follows stroke, cerebrovascular accident, brain or spinal chord trauma, myocardial infarct, physical trauma, drownings, suffocation, perinatal asphyxia, or hypoglycemic events. To reduce neurotoxic injury, the conantokins should be administered in a therapeutically effective amount to the patient within 24 hours of the onset of the hypoxic, anoxic or ischemic condition in order for the conantokins to effectively minimize the CNS damage which the patient will experience.

The conantokins are further useful for the treatment of Alzheimer's disease, senile dementia, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, Parkinson's disease, Huntington's disease, Down's Syndrome, Korsakoff's disease, schizophrenia, AIDS dementia, multi-infarct dementia, Binswanger dementia and neuronal damage associated with uncontrolled seizures. The administration of the conantokins in a therapeutically effective amount to a patient experiencing such conditions will serve to either prevent the patient from experiencing further neurodegeneration or it will decrease the rate at which neurodegeneration occurs. In addition, the conantokins can be administered in adjunct with conventional treatment agents to reduce the amount of such agents which need to be used.

The conantokins are also useful for treating chemical toxicity (such as addiction, morphine tolerance, opiate tolerance, opioid tolerance and barbiturate tolerance), anxiety, major depression, manic-depressive illness, obsessive-compulsive disorder, schizophrenia, mood disorders (such as bipolar disorder, unipolar depression, dysthymia and seasonal effective disorder), dystonia (movement disorder), sleep disorder, muscle relaxation, urinary incontinence, HIV infection and ophthalmic indications. In treating these conditions, a therapeutically effective amount of one or more conantokins is administered to a patient to completely treat the condition or to ease the effects of the condition. In addition, the conantokins are useful for memory/cognition enhancement (treating memory, learning or cognitive deficits), in which case a therapeutically effective amount of the conantokins is administered to enhance memory or cognition.

The conantokins are further useful in controlling pain, e.g., as analgesic agents, and the treatment of migraine, acute pain or persistent pain. They can be used prophylactically or to relieve the symptoms associated with a migraine episode, or to treat acute or persistent pain. For

these uses, the conantokins are administered in a therapeutically effective amount to overcome or to ease the pain.

The anticonvulsant effects of conantokins have been demonstrated in animal models. In rodents, conantokins are effective against supramaximal tonic extension seizures produced by maximal electroshock and threshold seizures induced by subcutaneous (s.c.) pentylenetetrazole or picrotoxin. As described in further detail below, Conantokin R was found to have an antiseizure activity greater than 400,000-fold higher than the standard commercial antiepileptic drug, valproic acid. In addition, Conantokin R was found to have a protective index at least eight times better than that of valproic acid. Conantokins are also effective against focal seizures induced by aluminum hydroxide injection into the pre- and post-central gyri of rhesus monkeys. Conantokins, when administered to patients with refractory complex partial seizures, may markedly reduce seizure frequency and severity. Thus, conantokins are useful as anticonvulsant agents. Moreover, the clinical utility of conantokins as a therapeutic agent for epilepsy may include generalized tonic-clonic and complex partial seizures.

The neuroprotective effects of conantokins have been demonstrated in laboratory animal models. Conantokins protected against hypoxic damage to the hippocampal slice *in vitro*. In neonate rats, conantokins reduced the size of cortical infarcts and amount of hippocampal necrosis following bilateral carotid ligation and hypoxia. Thus, conantokins are useful as neuroprotective agents. Whereas other anticonvulsants may exhibit neuroprotectant properties (Aldrete et al., 1979; Abiko et al., 1986; Nehlig et al., 1990), these effects often occurred only at high, clinically achievable doses associated with considerable toxicity (Troupin et al., 1986; Wong et al., 1986). In contrast, conantokins exhibit both anticonvulsant and neuroprotectant effects at doses well tolerated by animals and humans.

The analgesic or anti-pain activity of conantokins is demonstrated in animal models of pain and in animal models of persistent pain. In these models, conantokins are (a) effective in nerve injury model studies; (b) effective in reducing the tolerance to opiate analgesics after chronic administration and (c) effective in inhibiting activation of NMDA receptors and thereby inhibiting the release of Substance P by small-diameter, primary, sensory pain fibers. Thus, conantokins are useful as analgesic agents and anti-pain agents for the treatment of acute and persistent pain. The conantokins are also useful for treating addiction, morphine/opiate/opioid tolerance or barbiturate tolerance.

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The anti-neurodegenerative disease or neuroprotective activity of conantokins is demonstrated in animal models of Parkinson's disease. The conantokins are effective in reversing the behavioral deficits induce by dopamine depletion. The conantokins show behavioral potentiation, especially locomotor activity. The conantokins enhance the effect of L-DOPA in reversing the behavioral deficits induce by dopamine depletion. Thus, conantokins are effective neuroprotective agents and anti-neurodegenerative disease agents.

The effect of conantokins on muscle control is demonstrated in animals. At low doses, the conantokins are effective in hampering voiding at the level of the urethra. At higher doses, the conantokins are effective in eliminating all lower urinary tract activity. In the animal studies, it appears that the conantokins are more discriminatory in their inhibitory effects on striated sphincter than on bladder when compared with other NMDA antagonists. Thus, the conantokins can be dosed in such a way so as to selectively decrease bladder/sphincter dyssynergia, especially in spinal cord injured patients, and are therefore useful for treating urinary incontinence and muscle relaxation.

Pharmaceutical compositions containing a compound of the present invention as the active ingredient can be prepared according to conventional pharmaceutical compounding techniques. See, for example, *Remington's Pharmaceutical Sciences*, 17th Ed. (1985, Mack Publishing Co., Easton, PA). Typically, an antagonistic amount of active ingredient will be admixed with a pharmaceutically acceptable carrier. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., intravenous, oral, parenteral or intrathecally.

For oral administration, the compounds can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions or emulsions. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, suspending agents, and the like in the case of oral liquid preparations (such as, for example, suspensions, elixirs and solutions); or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations (such as, for example, powders, capsules and tablets). Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques. The active agent must be stable to passage through the gastrointestinal tract.

If necessary, suitable agents for stable passage can be use and may include phospholipids or lecithin derivatives described in the literature, as well as liposomes, microparticles (including microspheres and macrospheres).

For parenteral administration, the compound may dissolved in a pharmaceutical carrier and administered as either a solution of a suspension. Illustrative of suitable carriers are water, saline, dextrose solutions, fructose solutions, ethanol, or oils of animal, vegetative or synthetic origin. The carrier may also contain other ingredients, for example, preservatives, suspending agents, solubilizing agents, buffers and the like. When the compounds are being administered intracerebroventricularly or intrathecally, they may also be dissolved in cerebrospinal fluid.

The conantokins can also be administered in a cell based delivery system in which a DNA sequence encoding a conantokin is introduced into cells designed for implantation in the body of the patient, especially in the spinal cord region. Suitable delivery systems are described in U.S. Patent No. 5,550,050 and published PCT Application Nos. WO 92/19195, WO 94/25503, WO 95/01203, WO 95/05452, WO 96/02286, WO 96/02646, WO 96/40871, WO 96/40959 and WO 97/12635.

The conantokins are administered in an amount sufficient to antagonize the effects of excitatory amino acids or other agonists upon the NMDA receptor complex. The dosage range at which these conantokins exhibit this antagonistic effect can vary widely depending upon the particular disease or condition being treated, the severity of the patient's disease or condition, the patient, the specific conantokin being administered, the route of administration and the presence of other underlying disease states within the patient. Typically the conantokins exhibit their therapeutic effect at a dosage range from about 0.015 mg/kg to about 200 mg/kg, preferably from about 0.02 mg/kg to about 100 mg/kg of the active ingredient, more preferably from about 0.03 mg/kg to about 75 mg/kg of the active ingredient, and most preferably from about 0.03 mg/kg to about 50 mg/kg of the active ingredient. A suitable dose can be administered in multiple sub-doses per day. Typically, a dose or sub-dose may contain from about 0.1 mg to about 500 mg of the active ingredient per unit dosage form. A more preferred dosage will contain from about 0.5 mg to about 100 mg of active ingredient per unit dosage form.

For newly diagnosed patients with a seizure disorder and patients with seizure disorders for whom changes in drugs are being made, a relatively low dosage of drug is started and increased over a week or so to a standard therapeutic dosage. After about a week at such dosage, blood

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levels are obtained to determine the patient's pharmacokinetic response and, if appropriate, whether the effective therapeutic level has been reached. If seizures continue, the daily dosage is increased by small increments as dosage rises above the usual. Once seizures are brought under control, the drug should be continued without interruption at least one seizure-free year. At that time, discontinuation of the drug should be considered, since about 50% of such patients will remain seizure free without drugs. Patients whose attacks initially were difficult to control, those who failed a therapy-free trial and those with important social reasons for avoiding seizures should be treated indefinitely.

10

EXAMPLES

The present invention is described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below were utilized. The examples demonstrate the purification and chemical synthesis of conantokin R. Conantokin Oc, conantokin S1, conantokin L, conantokin Gm, conantokin Ca1, conantokin Ca2 and conantokin Qu are purified and synthesized in a similar manner. Conantokin derivatives and conantokin chimeras are made in conventional manner, such as chemically synthesizing the desired derivative or chimera.

20

EXAMPLE 1

Methods

Sequence analysis. All sequence analyses were performed on the Applied Biosystems Model 477A pulsed liquid phase automated protein sequencer using the Nonnal-1 cycles. Peptides were immobilized on a glass fiber filter treated with TFA, in the presence of 3 mg Polybrene and 0.2 mg NaCl. PTH-amino acid derivatives were analyzed by RP-HPLC with an on-line Applied Biosystems Model 120A analyzer, and identified by retention time.

Pyridylethylation. The peptide (20 to 300 pmoles) was dissolved in 50 μ l 0.25 M TRIS-HCl, 6 M in guanidine hydrochloride, and 2 mM in EDTA, pH 7.5. Reduction was then carried out by adding 2 μ l 10% mercaptoethanol, aq., and incubating at room temperature for 30 min. The peptide was then alkylated by adding 2 μ l of a 20% solution of 4-vinyl pyridine in ethanol

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and incubating the mixture at room temperature, in the dark, for 15 min. The solution was acidified prior to de-salting by RP-HPLC with 5 μ l of 10% TFA.

HPLC. Analytical and micro-preparative HPLC were performed on the Hewlett-Packard Model 1090M system equipped with a diode array detector, using a Brownlee Aquapore RP-300
5 C-8 column, 2.1 x 100 mm and a TFA/acetonitrile system.

Amino acid analysis. Hydrolysis was performed in 4 N methanesulfonic acid at 110° C for 24 hrs. The hydrolysates were then analyzed on a Perkin-Elmer HPLC-based amino acid analyzer utilizing automated post-column OPA derivatization and fluorescence detection. Separation of the amino acids was done on an Interaction A-111 column at 55° C.

10 Total synthesis. Chemical synthesis of conantokin R and its C-terminal amidated analog was carried out essentially as described for conantokin G by Rivier et al. (1987), and as described further below. Most fluorenylmethoxycarbonyl (Fmoc) amino acids were purchased from Bachem, Torrance, CA. Fmoc- γ - γ -di-t-bu- γ -carboxyglutamic acid (Gla) was synthesized as described by Rivier et al. (1987) Side chain protection of Fmoc amino acids included Glu(γ -
15 t-bu), Lys(ϵ -Boc), Arg(MTR), Cys(S-trityl) and Tyr(O-but).

EXAMPLE 2

Purification of Conantokin R

Specimens of *Conus radiatus* were collected by trawlers in Manila Bay. Venom ducts
20 were dissected, and crude venom was harvested and fractionated using reverse phase HPLC chromatography as described under Methods. The various fractions were examined for sleep-inducing activity. Several such fractions were identified, including a major fraction referred to as the "sleeper-I peptide." The sleeper-I peptide was purified to homogeneity.

The amino acid sequence of the sleeper-I peptide was determined by standard Edman
25 methods. Two sequencing runs were carried out on the native material. A number of blank positions were detected on the first run; because of the homology of the sequence obtained with previously characterized conantokinins T and G, we hypothesized that some of the blank positions might be γ -carboxyglutamate (Gla) residues which would be consistent with a small amount of Glu detected. In the second sequencing run, the peptide was first reduced and alkylated in
30 order to convert cysteines to the pyridylethylated derivative using 4-vinylpyridine. Two of the

blank positions (21 and 25) from the first sequencing run yielded pyridylethylated Cys. This suggested that the remaining blank positions (3, 4, 11, 15) might be Glu residues.

Mass spectrometry was also used to assess the status of the missing residues; electrospray mass spectrometry was used, yielding a monoisotopic mass (MH^+) at $m/z = 3097.6$.

5 This value is consistent with the amino acid sequence if all remaining positions were indeed γ -carboxyglutamate residues, with the two cysteine residues in disulfide linkage and a free carboxyl C-terminus (predicted monoisotopic mass is 3097.4).

Together, the data are consistent with the structure of the sleeper-I peptide shown in SEQ ID NO:4, wherein the Cys residues are in disulfide linkage. Because of the clear homology of
10 the peptide to conantokins G and T, this peptide was designated as conantokin R (for radius).

EXAMPLE 3

Synthesis of Conantokin R and Conantokin R Amide

The proposed sequence assignment above was directly confirmed by chemical synthesis.
15 Solid phase synthesis of both peptides on 3 g of the Fmoc-Pro-palkoxybenzylalcohol resin and 1 g of the 2,4-dimethoxybenzhydryl-amine resin (Rivier et al., 1987) was done manually (0.5-3.0 mmol of amino acid/g of resin). Monitoring of coupling/deblocking for each cycle was done using the Kaiser test. (Kaiser, 1970) Removal of the Fmoc group was effected by treatment with 0.1 M TBAF (tetrabutylammonium fluoride) in dimethylformamide (DMF) in the case of
20 the peptide acid and with two treatments (3 + 7) min of a 20% solution (v/v) of piperidine in DMF for the peptide amide. Resin washing was accomplished by repeated application of DMF and/or dichloromethane (DCM) and methanol (MeOH). Couplings were mediated by 1,3-diisopropylcarbodiimide (DIC) in DCM/DMF in the presence of 0.60.7 eq of 1-hydroxybenzotriazole (HOBt). Fmoc-Asn was incorporated into the peptide with the side chain
25 unprotected, in the presence of 0.6 to 2 equivalent of HOBt in dimethylsulfoxide (DMSO)-DCM/DMF. The crude peptides were purified using preparative HPLC techniques as previously described (Rivier et al., 1984). Peptides were dissolved in 0.1 M sodium borate buffer in the presence of 10 mM $CaCl_2$ in order to obtain sharp absorbances for CZE at pH 8.5 and HPLC at pH 7.4.

30 Conantokin R. Cleavage and deprotection of the peptide (2 x 2.0 g peptido-resin) was achieved by treatment with a freshly prepared mixture of trifluoroacetic acid, thioanisole, H_2O ,

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ethanedithiol and DCM (40:8:1:2:49) (20 ml/g) at 37° C for 7.25 hr. Trial cleavages of small amounts (10 mg of peptide resin had demonstrated that the peptide would be freed from the resin and all side chains deprotected, while the Glu would remain intact under these conditions. The peptide was precipitated from the cleavage solution and washed by the addition of tert-butyl methyl ether (5 x 100 ml) in centrifuge tubes. The solid was suspended in water (60 ml) and the suspension filtered. The tubes and resin were washed with 40% acetic acid. The extracts were immediately cooled, and then dissolved and diluted to 4 l (in the presence of 10 g ammonium acetate pH 4.3). The pH was adjusted to 7.8 with NH₄OH and the solution was allowed to slowly stir and air oxidize at 4° C for 3 d. The progress of oxidation was followed by the Ellman test and HPLC analysis as the oxidized product formed. After acidification of the solution to pH 5 with acetic acid, the solution (2x ≈1.5 l) was applied directly to a preparative HPLC cartridge. The gradient of acetonitrile applied to the preparative cartridge in 0.1% TFA was 23-35% in 1 hr, with a flow rate of 100 ml/min. Analysis of the generated fractions was achieved using isocratic conditions (24% acetonitrile in 0.1% TFA) on a 5 µm Vydac column. Peptide-containing fractions were then lyophilized to yield 120 mg. The powder was re-applied to the preparative cartridge in TEAP pH 2.3 and a gradient of acetonitrile 12-24% eluted the peptide. Desalting was carried out using an acetonitrile gradient from 23-35% in 0.1% TFA in 30 min, then re-application and elution using acetonitrile from 12-57% in 10 min. Purified fractions were pooled and lyophilized yielding conantokin R as the trifluoroacetate (36 mg). Results of the HPLC and capillary zone electrophoresis (CZE) analyses of this material are given in Tables 1 and 2, respectively. Amino acid analysis after acid hydrolysis gave the following ratios with expected values in parentheses: Asp (2), 1.96; Glu (5) including presence of four Glu residues, 4.91; Pro (1) 1.26; Gly (2), 1.95; Ala (5) 5.00, Cys (2), 1.65; Val (2) 1.97; Met (1), 0.88; Ile (1), .097; Leu (1), 1.01; Tyr (1), 0.98; Lys (3), 3.03; Arg (1), 0.98.

Conantokin R amide: Cleavage, deprotection and cyclization of the peptide (1.6 g peptido-resin) was achieved as described above. However, after acidification of the oxidized solution to pH 5 with acetic acid, the solution was passed through two columns packed with cation-exchange resin (2.5 x 7 cm) Bio-Rex 70 (H⁺ form). Eluent was checked by HPLC to verify absence of the peptide. The column was then washed with 1% aqueous acetic acid (250 ml) and the desired oxidized peptide was eluted with 50% aqueous acetic acid. Fractions were collected (5 ml) and tested with ninhydrin. Peptide-containing fractions were combined and

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diluted 5x with H₂O, shell frozen and lyophilized (yield 300 mg). The gradient of acetonitrile applied to the preparative cartridge in TEAP pH 2.25 was 15-30% in 1 hr, with a flow rate of 100 ml/min. Analysis of the generated fractions was achieved using isocratic conditions (21% acetonitrile in TEAP pH 2.25) on a 5 μ m Vydac column. Peptide-containing fractions were then re-applied to the preparative cartridge in TEAP pH 5.2 and the same gradient of acetonitrile applied. Desalting was carried out using an acetonitrile gradient from 9-36% in 0.1% TFA in 45 min. Purified fractions were pooled and lyophilized yielding conantokin R amide as the trifluoroacetate (12.5 mg). The purified material was analyzed by HPLC and capillary zone electrophoresis (see Tables 2 and 3). Amino acid analysis of the synthetic material was consistent with the conantokin R sequence. The synthetic material exhibited the same biological activity as the native material, and a mixture of synthetic and native material gave a single homogeneous peak on HPLC.

A conantokin R analog with an amidated C-terminus was also synthesized. This material did not co-elute with the native conantokin R, verifying that in the natural peptide, the C-terminal Pro residue had a free carboxyl group. Nevertheless, this material was also biologically active with similar activity shown by conantokin R. Electrospray mass spectrometry (MS) for conantokin R and conantokin R-amide, showed protonated molecular ion [MH]⁺ at m/z = 3097.6 and m/z = 3096.7, corresponding to the calculated monoisotopic peptide acid of 3097.4 and 3096.4, respectively. FAB MS was also performed for conantokin R and the spectrum showed an intact ion at m/z = 3098.4.

TABLE 2

HPLC Analysis of Conantokin R (A) and Conantokin R-Amide (B)

	Solvent System	Flow Rate	Gradient	Retention Vol (ml)	% Purity
25	A. TEAP pH 7.4/CH ₃ CN ^a	0.2	18-36% CH ₃ CN in 30'	2.0	85
	A. 0.1% TFA/CH ₃ CN ^b	2.0	6-42% CH ₃ CN in 45'	54.0	≈90
	B. TEAP pH 7.4/CH ₃ CN ^a	0.2	18-36% CH ₃ CN in 30'	2.3	90
30	B. TEAP pH 2.3/CH ₃ CN ^b	2.0	15-30% CH ₃ CN in 20'	31.0	90

^a UV monitoring at 214 nm, 0.12 absorbance unit at full scale. Column was Vydac (0.21 x 15 cm) packed with C₁₈ 5 μ m particles, 300 Å pore size.

35 ^b UV monitoring at 210 nm, 0.10 absorbance unit at full scale. Column was Vydac (0.46 x 25 cm) packed with C₁₈ 5 μ m particles, 300 Å pore size.

TABLE 3

CZE Analysis of Conantokin R (A) and Conantokin R-Amide (B)

	Buffer System	Voltage (kV)	Migration time (min)	% Purity
5	A. 0.1 M sodium borate in (85 H ₂ O:15 CH ₃ CN) pH 8.5 ^a	15	9.0	≈95
	A. 0.1 M phosphate pH 1.5 ^b	12	15.5	95
	B. 0.1 M sodium borate in (85 H ₂ O:15 CH ₃ CN) pH 8.5 ^a	15	8.4	≈90
10	B. 0.1 M phosphate pH 1.5 ^b	12	15.7	96

^a UV monitoring at 214 nm, 0.01 absorbance unit at full scale. Capillary was Beckman eCAP fused silica (75 µm x 60 cm). Temperature was maintained at 30° C.

^b UV monitoring at 214 nm, 0.01 absorbance unit at full scale. Capillary was Beckman eCAP fused silica (75 µm x 50 cm). Temperature was maintained at 30° C.

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EXAMPLE 4

Isolation of DNA Encoding Conantokins

DNA coding for the conantokins was isolated and cloned in accordance with conventional techniques using the general procedures and probes or primers set forth below.

Con G: The DNA was isolated using the toxin sequence degenerate probe DHOG108: CARGARAAYCARGARYT (SEQ ID NO:44) by Southern hybridization from a library of *C. geographus* DNA. The sequence of the DNA and its corresponding amino acid sequence are set forth in SEQ ID NO:45 and SEQ ID NO:46, respectively. The mature peptide sequence prior to Gla modification (residues 81 to 97 of SEQ ID NO:46) contains a Val at position 5 instead of Leu identified in the isolated peptide. The C-terminal GKR are processed to a C-terminal amide in the mature peptide.

Con R: The DNA was initially isolated using the probe DHOG424: CCYTNGCDATRTTYTC (SEQ ID NO:47) by Southern hybridization from a library of *C. radiatus* DNA. The full length clone was obtained by Southern Hybridization using the signal sequence probe DHOG450: GCCGTGCCTAGGATTA (SEQ ID NO:48). The sequence of the DNA and its corresponding amino acid sequence are set forth in SEQ ID NO:49 and SEQ ID NO:50, respectively. The mature peptide sequence prior to Gla modification corresponds to residues 81 to 107 of SEQ ID NO:50.

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Con Oc: The DNA was isolated using PCR with the signal sequence and 3' untranslated primers DHOG474: TGCTCGAATAAACATGAAAGATTTGGGGAA (SEQ ID NO:51) and DHOG475: TCTGCGATGCAACTGTACACGTATCTG (SEQ ID NO:52). The sequence of the DNA and its corresponding amino acid sequence are set forth in SEQ ID NO:53 and SEQ ID NO:54, respectively. The mature peptide sequence prior to Gla modification corresponds to residues 74 to 96 of SEQ ID NO:54. The C-terminal GR are processed to a C-terminal amide in the mature peptide.

Con Sl: The DNA was isolated using PCR with the signal sequence and 3' untranslated primers DHOG474 and DHOG475. The sequence of the DNA and its corresponding amino acid sequence are set forth in SEQ ID NO:55 and SEQ ID NO:56, respectively. The mature peptide sequence prior to Gla modification corresponds to residues 80 to 103 of SEQ ID NO:56.

Con L: The DNA was isolated using PCR with the signal sequence and 3' untranslated primers DHOG474 and DHOG475. The sequence of the DNA and its corresponding amino acid sequence are set forth in SEQ ID NO:57 and SEQ ID NO:58, respectively. The mature peptide sequence prior to Gla modification corresponds to residues 74 to 92 of SEQ ID NO:58. The C-terminal GK are processed to a C-terminal amide in the mature peptide.

Con Gm: The DNA was isolated using PCR with the signal sequence and 3' untranslated primers DHOG474e: GGAATTCAATAAACATGAAAGATTTGGGGAA (SEQ ID NO:59) and DHOG475E: GGAATTCGCGATGCAACTGTACACGTATCTG (SEQ ID NO:60). The sequence of the DNA and its corresponding amino acid sequence are set forth in SEQ ID NO:61 and SEQ ID NO:62, respectively. The mature peptide sequence prior to Gla modification corresponds to residues 74 to 92 of SEQ ID NO:62. The C-terminal GKR are processed to a C-terminal amide in the mature peptide.

Con Ca2: The DNA was isolated using PCR with the signal sequence and 3' untranslated primers DHOG474e and DHOG475e. The sequence of the DNA and its corresponding amino acid sequence are set forth in SEQ ID NO:63 and SEQ ID NO:64, respectively. The mature peptide sequence prior to Gla modification corresponds to residues 74 to 91 of SEQ ID NO:64. The C-terminal GK are processed to a C-terminal amide in the mature peptide.

Con Qu: The DNA was isolated using PCR with the signal sequence and 3' untranslated primers DHOG474e and DHOG475e. The sequence of the DNA and its corresponding amino

acid sequence are set forth in SEQ ID NO:65 and SEQ ID NO:66, respectively. The mature peptide sequence prior to Gla modification corresponds to residues 74 to 91 of SEQ ID NO:66. The C-terminal GKRR are processed to a C-terminal amide in the mature peptide.

On the basis of the peptide sequences for these conantokins, the consensus N-terminal peptide Met-Xaa₁-Leu-Tyr-Thr-Tyr-Leu-Tyr-Leu-Leu-Val-Xaa₂-Leu-Val-Xaa₃-Xaa₄ (SEQ ID NO:67), where Xaa₁ is His or Gln, Xaa₂ is Pro or Ser, Xaa₃ is Thr or Ala and Xaa₄ is Leu or Phe is derived. Primers and/or probes are made on the basis of this sequence and used alone or in combination with the primers and/or probes described above to isolate additional conantokin peptides from other species of *Conus*.

10

EXAMPLE 5

Specificity of Conantokin R for NMDA Receptor Subtypes

Conantokins G and T were previously shown to inhibit NMDA receptors in a variety of systems. The efficacy of conantokin R was compared to conantokin G using cloned NMDA receptor subunit combinations expressed in oocytes.

The NR1 subunits can be functionally expressed as a homomeric NMDA receptor complex in oocytes. From a comparison of the effects of conantokin R and conantokin G on such homomeric NR1 subunit complex, it is clear that while conantokin G inhibits both of the major splice variants tested, conantokin R is selective. At the concentrations tested, the peptide only inhibited the NR1.1B subtype, with no effect on the corresponding A subtype. Specifically, Conantokin R at a concentration of 3 μ M inhibited approximately 95% of the current produced by the NR1-1b/NR2B NMDA subtype in response to glutamate and glycine. The use of lower concentrations of Conantokin R gave a K_i of 0.14 μ M for the NR1-1b/NR2B subtype. The affinity of Conantokin R appears to be greater than 50-fold higher for this subtype than for the NR1-1b/NR2D combination, which was not affected even at 10 μ M peptide. Conantokin R was found not to inhibit the AMPA receptor GluR1 and kainate receptors (GluR6).

The effect of conantokins G and R on heteromeric complexes containing both NR1 and NR2 subunit combinations was also examined. In the combinations of NR1:NR2B examined, the currents being elicited by the presumptive heteromeric combination are much larger than when homomeric NR1 subunits are expressed. Conantokin R and conantokin G both inhibited such complexes if the B splice variant of the NR1 subunit was used. However, conantokin R

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proved to be selective for the B splice variant of the NR subunit, even in these heteromeric complexes, while conantokin G was not. The results indicate that conantokin R is a subtype-specific antagonist of the NMDA receptor, and has a preference for the B splice variant which contains an additional 21 amino acids.

5

EXAMPLE 6

In vivo Activity of Conantokins in
Frings Audiogenic Seizure Susceptible Mice

In vivo anticonvulsant activity of conantokins was analyzed in Frings audiogenic seizure
10 susceptible mice as described by White et al. (1992). The results for conantokin R are shown in
Tables 4, 5 and 6.

TABLE 4

Effect of Conantokin R on the Audiogenic Seizure
15 Susceptibility of Frings Mice Following i.c.v. Administration

	Dose (pmol i.c.v.)	# Protected / # Tested		# Protected / # Tested	
		15 min.	60 min.	15 min.	60 min.
	90	4 / 4	4 / 4	1 / 4	4 / 4
20	360	4 / 4	4 / 4	4 / 4	3 / 4

Ref: SW1:154

TABLE 5

Time Effect of Conantokin R Against Audiogenic Seizure
25 Susceptibility of Frings Mice Following i.c.v. Administration

			Time (hrs)					
		<u>Dose</u>	<u>1/4</u>	<u>1/2</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>Reference</u>
30	# Prot. / # Tested	9 pmol	2 / 4	2 / 3	3 / 4	1 / 4	0 / 4	SW1:155, 159
	# Toxic / # Tested	9 pmol	1 / 8	0 / 4	1 / 8	0 / 4	0 / 4	SW1:154, 160

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TABLE 6

Effect of Conantokin R on the Audiogenic Seizure
Susceptibility of Frings Mice Following i.c.v. Administration

	Dose (pmol, i.c.v.)	Seizure Score \pm S.E.M.	# Protected / # Tested (at 1 hr)	ED ₅₀ (pmol, i.c.v.)	# Toxic / # Tested (at 1/4 hr)	TD ₅₀ (pmol)
5	2.27	5 \pm 0	0 / 4			
10	4.50	4.4 \pm 0.62	1 / 8			
	9	2.75 \pm 0.86	4 / 8	9.00		
	1.8	1.125 \pm 0.58	7 / 8	(5.98 - 14.3)*		
	9	0 \pm 0	4 / 4		1 / 8	164
	18.0	--	--		4 / 8	(111 - 233)*
15	36.0	0 \pm 0	4 / 4		8 / 8	

* 95% confidence interval

Ref: SW1:153, 154, 159-161

20 Conantokin R yielded an effective dose (ED₅₀) of 9 pmol. The ED₅₀ for conantokin -T was 5.1 pmol (95% CI = 3.3 - 9.5 pmol). The ED₅₀ for conantokin G was 1.0 pmol (95% CI = 1.0-2.0 pmol). Furthermore, conantokin R yielded a toxic dose (TD₅₀) of 164 pmol. The dose required to elicit neurotoxicity was 18 times greater than the effective dose (TD₅₀/ED₅₀ - 164/9 = 18 = Protective Index, PI). The TD₅₀ for conantokin G was 28 pmol (95% CI = 22-35 pmol),
 25 yielding a protective index of 27. Moreover, the PI of 18 for conantokin R and 27 for conantokin G exceeds that of other anti-seizure medications tested in this model. The therapeutic dose of typical anti-seizure medications is close to the toxic dose (typical PI = 2-3). Since the protective index is high for conantokin R and conantokin G, these peptides will be better tolerated than previous anti-convulsant agents.

30 Similar results are obtained for conantokin Sl, G, T, L, Gm, Ca2 and Qu and analogs of these peptides in which the γ -carboxyglutamic acid residues other than at positions 3 and 4 are substituted by other amino acid residues, including Ser, Ala, Glu and Tyr. These results are consistent with the finding that several Con G synthetic analogs possess high affinities for non-competitive inhibition of polyamine enhanced [³H]MK-801 binding (Zhou et al., 1996).

EXAMPLE 7

Comparison of *In vivo* Activity of Conantokins and
Standards in Frings Audiogenic Seizure Susceptible Mice

The anticonvulsant profile of several conantokins and the standards dizocilpine (MK-801), ifendropil and valproic acid was determined using Frings audiogenic seizure-susceptible mice (25-30 g body weight) obtained from the house colony of the University of Utah. All intracerebroventricularly (i.c.v.) injections were made free-handed into the lateral ventricle (approximately 1 mm lateral, 1 mm anterior from bregma and to a depth of 3 mm from the surface of the skull) of awake mice with a 10 μ l Hamilton syringe. Varying doses of the compounds were tested. At the predetermined time of peak anticonvulsant effect, individual mice were placed into a round Plexiglas chamber (diameter, 15 cm; height, 18 cm) pitted with an audio transducer (Model A5-ZC; FET Research & Development, Salt Lake City, Utah) and exposed to a high intensity sound stimulus (110 decibels, 11 KHz) for 25 seconds. Animals not displaying tonic forelimb or hindlimb extension were considered protected. The effect of the test compounds on motor performance was assessed by the rotorod test (Dunham and Miya, 1957). For this procedure, mice were tested for their ability to maintain balance on a rotating (6 rpm) knurled Plexiglas rod (1 inch diameter) for one minute. Mice unable to maintain balance in three successive trials during the test period were considered toxic. The median effective dose (ED_{50}) and the median toxic dose (TD_{50}) was calculated by probit analysis (Finney, 1971). For these studies, the dose of each test substance was varied between the limits of 0 and 100% protection and toxicity. The protective index (PI) is TD_{50}/ED_{50} . The results are shown in Tables 7 and 8. The time-dependent inhibition of audiogenic seizures by Con R following i.c.v. administration is shown in Fig. 1. The ability of conantokins (Con R (◆), Con T (■) Con G (○)) to block audiogenic seizures in a dose-dependent manner following i.c.v. administration is shown in Fig. 2. The dose-dependent reduction in seizure severity following i.c.v. administration for conantokins (Con R (◆), Con T (■) Con G (○)) is shown in Fig. 3. The dose-dependent blockage of audiogenic seizures by Con R at non-toxic doses is shown in Figure 4. Protection (■) and impairment (○) are shown with an ED_{50} of 9 pmol and a TD_{50} of 164 pmol.

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TABLE 7

Comparative Anticonvulsant Efficacy, Minimal Motor Impairment and
Protective Index of Conantokins R, T, and G and Ifenprodil Following I.C.V. Administration

5	Test Substance	Time of Test ^a (min)	nmols. i.c.v.		
			ED ₅₀ ^b	TD ₅₀ ^b	P.I.
	Con R	60, 15	0.013 ^c (0.0083 - 0.020)	0.228 ^d (0.154 - 0.323)	17
10	Con T	30, 15	0.017 (0.011 - 0.032)	0.228 (0.154 - 0.323)	13
15	Con G	30, 15	0.0035 (0.002 - 0.005)	0.094 (0.073 - 0.116)	27
	Ifenprodil	5, 5	~25	<25	<1

^a First time, ED₅₀; Second time, TD₅₀

20 ^b 95% confidence interval in parentheses

^c The values for ED₅₀ and TD₅₀ for Con R, Con T and Con G are the raw data. These numbers are multiplied by 0.72 for Con R and by 0.3 for Con G and Con T to obtain values corrected for peptide content. The PI numbers do not change.

25

TABLE 8

Comparative Anticonvulsant Efficacy, Minimal Motor Impairment and Protective
Index of Conantokin R, MK801, Ifenprodil and Valproic Acid Following I.C.V. Administration

30	Test Substance	Time of Test ^a (min)	nmols. i.c.v.		
			ED ₅₀ ^b	TD ₅₀ ^b	P.I.
	Con R	60,15	0.013 ^c (0.0083 - 0.020)	0.228 ^d (0.154 - 0.323)	17
35	MK801	5,5	0.641 (0.415 - 0.933)	1.227 (0.639 - 4.532)	1.9
	Valproic Acid	5,5	5644 (3707 - 7759)	>6000 <12,000	1.1-2.2
40	Ifenprodil	5,5	~25	<25	<1

^a First time, ED₅₀; Second time, TD₅₀

^b 95% confidence interval in parentheses

45 ^c The values for ED₅₀ and TD₅₀ for Con R are the raw data. These numbers are multiplied by 0.72 for Con R to obtain values corrected for peptide content. The PI number does not change.

EXAMPLE 8

In vivo Activity of Conantokins in CF No. 1 Mice

In vivo anticonvulsant activity of conantokins R, Sl, G, T, L, Gm, Ca2 and Qu are analyzed in CF No. 1 mice as described by White et al. (1995), using the maximal electroshock, subcutaneous pentylenetetrazole (Metrazol) seizure threshold and threshold tonic extension test. Each of the conantokins tested are found to have anticonvulsant activity. Specifically, the activity of Conantokins R and G in this model animal are shown in Table 9.

TABLE 9

Anticonvulsant Efficacy, Minimal Motor Impairment and
Protective Index of Conantokins G and R Following I.C.V. Administration

Test Substance	Frings Audiogenic Mice			CF #1 Mice		
	ED ₅₀ ^a	TD ₅₀ ^a	P.I. ^b	MES ED ₅₀ ^a	TD ₅₀ ^a	P.I. ^b
Con G	0.0035 ^c (0.002 - 0.005)	0.094 ^c (0.073 - 0.116)	27	0.026 (0.013 - 0.038)	0.066 (0.048 - 0.091)	2.5
Con R	0.013 (0.0083 - 0.020)	0.228 (0.154 - 0.323)	17	0.083 (0.029 - 0.117)	~0.300	3.6

^a nmols; 95% confidence intervals in parentheses

^b Protective Index (TD₅₀/ED₅₀)

^c The values for ED₅₀ and TD₅₀ for Con R and Con G are the raw data. These numbers are multiplied by 0.72 for Con R and by 0.3 for Con G to obtain values corrected for peptide content. The PI numbers do not change.

EXAMPLE 9

In vivo Activity of Conantokin T in Frings Audiogenic
Audiogenic Seizure-Susceptible Mice Following I.C.V. Administration

In vivo anticonvulsant activity of Con T was analyzed in Frings audiogenic seizure-susceptible mice as described above except that the peptide was administered intravenously (IV) at 12 mg/kg. The peptide was administered to naive mice and pre-stimulated mice. The mice were dosed i.v. and stimulated at the indicated time intervals and the protection was measured. The pre-stimulated mice were stimulated at 1 minute as a pre-stimulation and then stimulated at the indicated time intervals. The results are shown in Tables 10 and 11. No animals exhibited behavioral toxicity at this dose, as determined by the rotarod test as described above.

TABLE 10

Anticonvulsant (Frings Audiogenic Seizure-Susceptible Mouse Model)
Activity of Conantokin T Following Intravenous (IV) Administration: Naive Animals

Time of Test (min)	Con-T #Protected/ #Tested	% Protected	Saline Control #Protected/ #Tested	% Protected
1				
10	0/9	0%	0/5	0%
20	1/2	50%	0/2	0%
30	2/6	33%	0/5	0%
60	4/6	67%		
240	1/6	17%		

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TABLE 11

Anticonvulsant (Frings Audiogenic Seizure-Susceptible Mouse Model) Activity
of Conantokin T Following Intravenous (IV) Administration: Pre-Stimulated Animals

10

Time of Test (min)	Con-T #Protected/ #Tested	% Protected	Saline Control #Protected/ #Tested	% Protected
1	1/2	50%	0/2	0%
10				
20	1/2	50%	0/2	0%
30	3/5	60%		
60	6/7	86%	6/12	50%
240	6/11	55%	0/4	0%

Table 10 shows 67% protection for the naive animals at 60 minutes following the i.v. dose of Conantokin T. The pre-stimulation sometimes results in erratic protection which may be due to compromising the blood-brain-barrier, thus, allowing CNS penetration by compounds that otherwise would not penetrate. Alternatively, the result in the pre-stimulated animals could

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be due to making the animals refractory to subsequent seizures. Nevertheless, the present experiment demonstrates the bioavailability of Conantokin T, since it protected the naive animals following i.v. dose. Similar results were obtained with Conantokin G.

EXAMPLE 10

In vivo Activity of Conantokin G in Frings Audiogenic
Seizure-Susceptible Mice Following I.C.V. Administration

In vivo anticonvulsant activity of conantokin G was analyzed in Frings audiogenic seizure susceptible mice as described above, except that the peptide was administered i.c.v. at 0.0038 nmol or 0.0056 nmol. Table 12 shows the time to onset of the anticonvulsant activity of Con G following i.c.v. administration.

TABLE 12

Time to Onset of Anticonvulsant Activity of Conantokin G: Seizure
Protection Following I.C.V. Administration to Frings Audiogenic Mice

Test Substance	Dose nmol, i.c.v.	Percent Protection (at time of test, min)					
		1	2	3	4	5	60
Con G ¹	0.0038	0	0	0	100	100	87.5
Con G ²	0.0056	25	50	100	-	-	100

¹N = 16; ²N = 8

At a Con G dose of 0.038 nmol, seizure protection was observed in 100% of the animals tested at four minutes. As a control, at the same dose of Con G, 87.5% of the animals were protected at 60 minutes. Moreover, at a Con G dose of 0.0056 nmol, seizure protection was observed in 25% of the animals tested at one minute, 50% at two minutes and 100% at three minutes. As a control, at the same dose of Con G, 100% of the animals were protected at 60 minutes. No animals exhibited behavioral toxicity (rotorod minimal motor impairment) at the doses of Con G and the times tested. Thus, Con G elicits a very rapid time to onset (within one to three minutes) of anticonvulsant activity, with high potency and low behavioral toxicity, following i.c.v. administration to Frings audiogenic mice.

A dose-response of the anticonvulsant activity of Con G at one and three minutes following i.c.v. administration in this model is shown in Figure 5. These data demonstrate that the median effective dose (ED_{50}) for anticonvulsant activity of Con G at one and three minutes was 0.023 nmol 0.004 nmol, respectively.

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EXAMPLE 11

In vivo Activity of Conantokin G in Frings Audiogenic
Seizure-Susceptible Mice Following I.C.V. Administration

In vivo anticonvulsant activity of Conantokin G was analyzed in Frings audiogenic seizure susceptible mice as described above with i.c.v. administration. Table 13 compares the median effective dose (ED_{50}), the median toxic dose (TD_{50}), rotorod minimal motor impairment and protective index ($PI = TD_{50}/ED_{50}$) of Con G at one, three and thirty minutes following i.c.v. administration. In these studies, all rotorod minimal motor impairment tests were performed at 30 minutes.

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TABLE 13

Time to Onset of Anticonvulsant Activity of Conantokin G: Efficacy, Minimal Motor Impairment and Protective Index Following I.C.V. administration to Frings Audiogenic Mice

	Test Substance	Time of Test (min)	Dose (nmol, i.c.v.)		
			ED_{50}	TD_{50}	PI
20	Con G	1 ^{1,2}	0.023	0.028 (0.022-0.035) ³	1.2
25	Con G	3	0.004 (0.003-0.004)	0.028 (0.022-0.035)	7.8
30	Con G	30	0.001 (0.001-0.002)	0.028 (0.022-0.035)	27

¹ N = 8 animals/group

² No animals exhibited minimal motor impairment at indicated doses at the time of test.

³ 95% confidence interval in parentheses.

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At one minute following Con G administration, the ED_{50} , TD_{50} and PI for anticonvulsant activity were 0.023 nmol, 0.028 nmol and 1.2, respectively. At three minutes following Con G

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administration, the ED₅₀, TD₅₀ and PI for anticonvulsant activity were 0.004 nmol, 0.028 nmol and 7.8, respectively. At 30 minutes following Con G administration, the ED₅₀, TD₅₀ and PI for anticonvulsant activity were 0.001 nmol, 0.028 nmol and 27, respectively. These data clearly show that the time to onset of Con G anticonvulsant activity following i.c.v. administration to Frings audiogenic mice was very rapid (within one to three minutes) with very low behavioral toxicity compared to prototypical antiseizure drugs in testing or on the market.

EXAMPLE 12

In vivo Phencyclidine-Like Behavioral Effects
of Conantokin G Following I.C.V. Administration

The *in vivo* phencyclidine-like behavioral effects of Con G was assessed by the elevated platform test as described by Evoniuk et al. (1991). The platform test is a rapid method for evaluating the behavioral effects of phencyclidine-like dissociative anesthetics in mice. At 15 minutes following a Con G dose of 0.0225 nmol (i.c.v.) to mice, no drug-induced falls from the elevated platform were observed. Alternatively, as a control, a 44.5 nmol dose of MK 801 (dizocilpine) elicited 87.5% drug-induced falls from the elevated platform. Thus, Con G does not induce phencyclidine-like behavioral effects in mice. The results are shown in Table 14.

TABLE 14

Absence of Phencyclidine-Like Behavioral Effects Using
Conantokin G Compared to MK 801: Activity in the Elevated Platform Test

<u>Test Substance</u>	<u>Maximum Dose Tested (nmol)</u>	<u>Percent Drug-Induced Falls from Elevated Platform</u>
Con G	0.0225 ¹ (i.c.v.)	0
MK 801	44.5 ² (i.p.)	87.5
MK 801	118.6 (i.p.)	100
H ₂ O	50 μ l (i.p.)	0

¹ ED₅₀, TD₅₀, PI as noted in Example 11.

² Dose used in these studies was the same as the minimum effective dose that induced $\geq 50\%$ of animals to fall from the elevated platform in Evoniuk et al. (1991).

EXAMPLE 13

Comparison of Modes of Administration of Conantokin G

In vivo anticonvulsant activity of conantokin G when administered i.c.v., i.v. or p.o. was analyzed in Frings audiogenic mice as described above. The ED₅₀ at 30 minutes was determined to be 0.048 nmol/kg for the i.c.v. administration and 702 nmol/kg for the i.v. administration. The 95% confidence interval for these values are 0.027 - 0.072 for i.c.v. administration and 341 - 1246 for i.v. administration. Figure 6 shows the dose-dependent inhibition of audiogenic seizures for i.c.v. and i.v. administration of Con G. The time-dependent inhibition of audiogenic seizures by Con G when administered i.v. (2650 nmol/kg) or p.o. (6623 nmol/kg) is shown in Figures 7 and 8, respectively.

EXAMPLE 14

Comparison of *In Vivo* Activity of Conantokin G with Prototype Antiepileptic Drugs

The *in vivo* anticonvulsant activity of Con G was compared to the anticonvulsant activities of several prototype antiepileptic drugs (AED) with i.c.v. administration in Frings audiogenic mice, as described above. The results are shown in Table 15. The protective index (PI) of Con G was significantly higher than the PI for the other drugs tested.

TABLE 15

Effect of Conantokin G Compared to Prototype Antiepileptic Drugs (AED) on
Audiogenic Seizure-Susceptibility Following i.c.v. Administration to Frings Mice

	Prototype AEDs	Time of Test (min)*	ED ₅₀ nmol	TD ₅₀ nmol	PI
5	Con G	30, 15	0.001 (0.001-0.002)	0.028 (0.022-0.035)	27
10	Con G	3, 15	0.004 (0.003-0.004)	0.028 (0.022-0.035)	7.8
	Phenobarbitol	5, 15	145 (105-186)	68.8 (42.9-93.5)	0.5
	Valproic Acid	5, 5	566 (3707-7759)	>6000- <12,000	1.1-2.2
15	Lamotrigine	30, 15-60	146 (101-195)	>290 limit of solubility	>2.0
	Felbamate	30, 15-60	5/8 protected at 525 4/8 protected at 630	no marked toxicity up to 630	unable to determine
20	Topiramate	15-240, 15-240	>150 nmol	no marked toxicity limit of solubility	unable to determine

TD₅₀ = rotorod performance, minimal motor impairment measure of behavioral toxicity

25 95% confidence interval in parenthesis

PI = Protective Index (PI = TD₅₀/ED₅₀)

* = 1st time ED₅₀; 2nd time TD₅₀

30

EXAMPLE 15

In Vivo Activity of Conantokin G in Pentylenetetrazole-Induced Threshold Seizure Model

The *in vivo* activity of Con G was analyzed using timed intravenous infusion of pentylenetetrazole (White et al., 1995). At time to peak effect, the convulsant solution (0.5% pentylenetetrazole in 0.9% saline containing 10 U.S.P. units/ml heparin sodium) is infused into
 35 the tail vein at a constant rate of 0.34 ml/min. The time in seconds from the start of the infusion to the appearance of the first twitch and the onset of clonus is recorded for each drug treated or control animal. The times to each endpoint are converted to mg/kg of pentylenetetrazole for each mouse, and mean and standard error of the mean are calculated. The results are shown in Table 16. Administration of Con G i.c.v. at 18.75 pmol elevates the i.v. pentylenetetrazole seizure
 40 threshold.

TABLE 16

Conantokin G Elevates i.v. Pentylenetetrazole (PTZ) Seizure Threshold

Treatment	Dose, pmol	PTZ, mg/kg	
		First Twitch	Clonus
Control	0	29.9 ± 2.5	41.9 ± 4.8
Conantokin G	18.75	47.3 ± 7.8	75.8 ± 11.1

N = 7 (Control), 8 (Conantokin G)

EXAMPLE 16

In Vivo Activity of Conantokin G in Parkinson's Disease Animal Model

The anti-Parkinsonian potential of conantokin G was examined in rats with unilateral lesions of the nigrostriatal dopamine system. The unilateral lesions are created by local infusion of the neurotoxin 6-hydroxydopamine (6-OHDA) into the right substantia nigra of anesthetized rats. The rats recovered for two weeks at which time they are anesthetized and guide cannulae implanted into the brain, ending in the right lateral ventricle. The guide cannulae are kept patent with a stylet placed in the guide cannula. One week later, the rats are placed in a cylindrical Plexiglas® cage, the stylet is removed, and an infusion cannula is inserted into the guide. The infusion cannula is attached to a syringe on an infusion pump which delivered conantokin G (0.5 mM or 5.0 mM) or control vehicle at a rate of 1 µl/min for a total injection of 2 µl (1 nmol/2µl). Fifteen minutes after the injection of conantokin G, L-Dopa (4 mg/kg ip) is injected. The number of full rotations contralateral and ipsilateral to the dopamine-depleted hemisphere is then counted for 2 minutes, every 10 minutes, for 2 hours. A video of the rats is also made to follow the behavioral potentiation of the treatment. The results are shown in Figs. 9 and 10. These results show that there is clear potentiation of the L-Dopa activity with Con G. The video showed that the behavioral potentiation by Con G is very dramatic, especially the locomotor activity. The ability to elicit contralateral rotation in this animal model leads to the conclusion that the tested compounds reverse the behavioral deficits induced by dopamine depletion. In addition to the above tests, the *in vivo* activity of Con G in combination with SKF 38393 was compared with that of SKF 38393 alone. The results are shown in Figs. 11-16. The combination of Con G and SKF 38393 demonstrated increased activity.

EXAMPLE 17

Regulation of Striatal Output Pathways by NMDA-2B Receptors

Experimental Parkinsonism results in altered functional activity of striatal output pathways. Neurons projecting to the globus pallidus (indirect pathway) become overactive, resulting in increased inhibition of cortical regions involved in movement and movement initiation via processing through the basal ganglia-thalamo-cortical loop. Treatment with non-selective NMDA antagonists (Klockgether and Turski, 1990) or lesions of the glutamergic neurons of the subthalamic nucleus alleviate Parkinsonian symptoms in experimental models (Bergman et al., 1990). The identification of multiple subtypes of NMDA receptor subunits and their differential expression throughout the basal ganglia nuclei offers the potential of altering glutamatergic transmission with specific nuclei. Of the primary basal ganglia nuclei, the NR2B subunit is expressed almost exclusively in their striatum (Standaert et al., 1994).

The recent discovery of a class of conantokins with remarkable selectivity for NMDA receptors expressing the NR2B subunit offers a unique pharmacological tool for the investigation of the role of this NMDA receptor subtype in the regulation of basal ganglia circuits, and its potential as a target for the treatment of movement disorders such as Parkinsonism. Con R will be administered i.c.v. alone or in combination with SKF 38393 in unilateral 6-hydroxydopamine-lesioned rats, or in combination with eticlopride in unlesioned rats, to precisely examine the role of NR2B receptors on immediate early gene induction in striatonigral and striatopallidal neurons which specifically express dopamine D1 or D2 receptors, respectively

EXAMPLE 18

In Vivo Activity of Conantokin G in Animal Model of Urinary Incontinence

Female Wistar rats are anesthetized with urethane and, following tracheotomy (for ventilation after skeletal muscle paralysis) and jugular and carotid cannulation (for drug delivery and blood pressure recording, respectively), laminectomies are performed at C7-T2 and T11-S1 through a midline dorsal incision. The back is temporarily closed and the animal is placed abdomen up. A midline incision is made from the sternum to the pubis. The ureters are isolated, ligated and cut proximally, and saline soaked gauze wicks are positioned at the cut end to exit the abdominal incision for urine elimination.

A double lumen urethral catheter is passed through a cystotomy at the dome of the bladder and seated in the urethral opening at the level of the internal sphincter. A second, single lumen catheter is positioned, through its own cystotomy, into the bladder. Both catheters are tied in with suture, and connected to pressure recording transducers and filling/perfusion syringes via three-way stopcocks.

Following closing of the abdomen, the rat is placed onto its abdomen and the back incision reopened and sutured to a metal ring to form a pocket for oxygenated Krebs solution. The spinal cord is cut at C7 and L3. Silver wire electrodes are introduced into the cut ends at C7 (inserted caudally) and L3 (inserted rostrally) for sympathetic preganglionic stimulation. Spinal roots L6 and S1 are placed on a hook electrode for stimulation of parasympathetic preganglionic axons. A continuously oxygenated Krebs solution fills the dorsal pocket and bathe the spinal cord and roots.

Simultaneous, independent electrical stimulation is delivered a low levels to both sets of preganglionic pathways and independent adjustment of stimulus parameters is made to achieve maximal responses from the blood pressure (sympathetic preganglionic stimulus driven) and the bladder (parasympathetic preganglionic driven).

Conantokins are introduced intrathecally and changes in blood and bladder pressure responses under constant drive are monitored, recorded and taped. Control studies are made with hexamethonium bromide and all studies finish with hexamethonium bromide administration.

Conatokin G was administered intrathecally at 0.3 nmol, 3.0 nmol and 30 nmol. The 0.3 nmol dose had no effect. The 3.0 nmol dose had no effect on bladder contraction amplitude, but increased frequency. This means that voiding was hampered at the level of the urethra. The 30 nmol dose eliminated all lower urinary tract activity. Similar effects were seen for conantokin T.

Further effects of Con G and Con T on bladder contraction amplitude and on EUS EM G activity are shown in Figs. 17 and 18. The conantokins appear to be more discriminatory in their inhibitory effect on striated sphincter than on bladder, compared to other NMDA antagonists. Thus, it is possible to dose the conantokins in such a manner to selectively decrease bladder/sphincter dyssynergia in spinal cord-injured patients.

EXAMPLE 19

Biological Activity of Conantokin Peptide
Derivatives and Conantokin Peptide Chimeras

Several conantokin peptide derivatives and conantokin peptide chimeras were prepared
 5 using conventional techniques and their inhibitory activity was measured using the spermine-
 stimulated [³H]MK-801 binding assay as described by Zhou et al. (1996). The results are shown
 in Table 17. A value for IC₅₀ of < 100 μM has been found to have activity in the *in vivo* assays
 described herein.

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TABLE 17

Inhibitory Activity of Con G Derivatives and Chimeras

	Test Substance	Sequence					IC ₅₀ (μM)
15	Con G	G	E	γ	γ	L	0.195
	A ³						9.1
	Y ³						0.96
	S ³						3.6
	S _p ³						0.9
20	A ⁴						≥ 20
	S ⁴						≥ 20
	S _p ⁴						≥ 20
	E ⁴						≥ 20
	A ⁷						0.045
25	Y ⁷						0.96
	S ⁷						0.25
	S _p ⁷						1.2
	A ¹⁰						1.1
	S ¹⁰						1.8
30	S _p ¹⁰						0.56
	A ¹⁴						0.16
	S ¹⁴						0.16
	S _p ¹⁴						1.59
	A ^{7,Y¹⁰}						0.079
35	A ^{7,10,14}						0.088
	E ^{7,10,14}						≥ 20
	A ^{2,7,10,14}						ND
	A ^{3,7,10,14}						ND
	A ^{4,7,10,14}						ND
40	Y ³ ,S ⁴ ,A ^{7,10,14}						ND

TABLE 15 (continued)

Inhibitory Activity of Con G Derivatives and Chimeras

Test	Substance	Sequence	IC ₅₀ (μM)
5	Con G	G E γ γ L Q γ N Q γ L I R γ K S N	0.195
	(1-10)	Δ Δ Δ Δ Δ Δ Δ Δ Δ Δ Δ Δ Δ Δ	ND
	(A ^{7,10,14})5-17	Δ Δ Δ Δ A A A	ND
	5-17	Δ Δ Δ Δ	ND
10	(A ^{10,14})6-17	Δ Δ Δ Δ Δ A A	ND
	(A ¹⁴)12-17	Δ Δ Δ Δ Δ Δ Δ Δ Δ Δ A	ND
	12-17	Δ Δ Δ Δ Δ Δ Δ Δ Δ Δ	ND
	(A ^{7,10,14})1-16	A A A Δ	0.037
	(A ^{7,10,14})1-15	A A A Δ Δ	0.034
15	(A ^{7,10,14})1-14	A A A Δ Δ Δ	0.232
	(A ^{7,10})1-13	A A Δ Δ Δ Δ	0.253
	(A ^{7,10})1-12	A A Δ Δ Δ Δ	0.30
	(A ^{7,10,14})2-17	Δ A A A	inactive
	(A ^{7,10,14})3-17	Δ Δ A A A	inactive
20	(A ^{7,10,14})4-17	Δ Δ Δ A A A	inactive
	(A ^{7,10,14})5-17	Δ Δ Δ Δ A A A	inactive
	(A ^{7,10,14})6-17	Δ Δ Δ Δ A A A	inactive
	Con G(T) ₂	Y Q K M L	ND
	Con G(L) ₄		D A V N
25	Con G(T) ₂₋₃	Y Q K M L N L	ND
	Con G(R) ₂₋₃	V A K M A A A	ND

30 All derivatives have an amide C-terminal. γ is γ-carboxyglutamic acid. S_p is phosphoserine. Δ indicates a deletion of residue. ND is not determined.

EXAMPLE 20

Biological Stability of Conantokins

35 The stability of Con G, Con T, Con R and several synthetic derivatives was determined in different biological media comprising serum (FS, 20%) and homogenates of liver (FL, 5%), kidney (FK, 25%) and brain (FB, 25%) from Frings audiogenic mice. The stability of Con G in normal saline as pH ≈ 6 was also examined. The biological fluid was diluted to appropriate percentage with RPMI-1640 cell culture media and preincubated for 15 min at 37° C in water bath. Peptides (1 mg/ml stock) were added to the biological media to a concentration of 50 μg/ml and incubated at 37°C. Peptide aliquots (100 μl) were removed at timed intervals and

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added to 200 μ l of ice cold 6% trifluoroacetic acid (TFA) and chilled on ice at least 15 min. Supernatant was recovered following centrifugation at 14,000 x g for 3 min and frozen until analysis. Two hundred μ l sample was injected into a HPLC system comprising 2 Dynamax Model SD-300 solvent delivery pumps, Rheodyne injection port, 5 ml injection loop, mixer and bubble suppression unit (Dynamax), Vydac T18TPS4 protein and peptide C₁₈ column with precolumn and prefilter, Dynamax absorbance detector Model UV-D11 and Dell Pentium computer with Dynamax chromatography software. The mobile phase used 0.1% TFA/H₂O as Buffer A and 0.1% TFA/acetonitrile as Buffer B. The following gradient was utilized:

	Time (min)	Flow Rate (ml/min)	% Buffer A	% Buffer B
10	0	1	95	5
	3	1	95	5
	25	1	45	55
	27	1	45	55
15	29	1	5	95
	33	1	5	95

The peak height and retention time was measured with Dynamax software. The results are shown in Figs. 19 and 20. Con G, [Ala⁷]Con G, Con-T and Con R were stable in all biological media for up to 4 hours. The derivatives ECon G and ECon R (Gla residues replaced by Glu residues) demonstrated rapid degradation in FL ($T_{1/2}$ = 25.5 sec and 2.4 min, respectively). The derivative ECon G demonstrated rapid degradation in FB ($T_{1/2}$ = 1.6 min). The results for the stability of Con G in normal saline is shown in Fig. 21. Approximately 60% of the Con G is left after 14 days.

EXAMPLE 21

In vivo Activity of Conantokins on Preliminary Screening

The conantokins were originally described as producing a sleep-like state in mice younger than two weeks old (Haack et al., 1990). The conantokins are screened for biological activity using a modified form of this test. Several measures are assessed to measure the degree of the sleep-like state. Catalepsy, sleepy and righting reflex tests are designed to quantify the "sleep-like" behavior noted by Rivier et al. (1987). In all three assays, young (<2 weeks old) mice are given a free-hand i.c.v. dose (100 pmol/g in 10 μ l) of a compound. Behaviors are assessed at 30 and 60 minutes post-injection.

Catalepsy Test: Young mice are positioned such that the two front paws are placed on an overturned Petri dish. A mouse is considered cataleptic if it fails to remove its paws in a 30 second period. *Sleepy Test:* Young mice are observed without interference. If no activity is noted, the animal is nudged with a gloved finger. A mouse is considered "sleepy" if it makes no attempt to move away from the finger. *Righting Reflex:* Young mice are positioned on their backs with legs in the air. A mouse is considered to have lost its "righting reflex" if it fails to right itself (return to its normal position with paws on the floor) in 10 seconds, thus "3/4" means three out of four mice lost righting reflex. In general, the ability of relatively high doses of conantokins to induce a sleep-like state is correlated with affinity and efficacy in the spermine-stimulated [³H]MK-801 binding assay described in Example 19. The results are shown in Table 18.

TABLE 18

Conantokin dose ¹	Catalepsy Test ^a		Sleepy Test ^b		Righting Reflex ^c	
	30 min	60 min	30 min	60 min	30 min	60 min
Con G	4/4	2/4	4/4	4/4	2/4	2/4
Con T	3/4	1/4	4/4	4/4	2/4	2/4
Con R	2/3	2/3	2/3	2/3	2/3	2/3
Con L	4/4	3/4	4/4	4/4	2/4	3/4
Con Oc	0/3	0/3	2/3	0/3	0/3	0/3
Saline	0/2	0/2	0/2	0/2	0/2	0/2

¹ ~ 2 week old mice, 5-7 g, sex not checked, i.c.v. dose of 100 pmol/g (600 pmol in 12 µl) or 12 µl of normal 0.9% saline

^a positive if mice leave forepaws on Petri dish for >30 s

^b positive if mice do not respond by moving away or sniffing after a finger poke

^c positive if mice fail to right themselves in <10 s

EXAMPLE 22

In vivo Activity of Conantokins in Pain Models

The anti-pain activity of conantokin is shown in several animal models. These models include the nerve injury model (Chaplan, et al., 1997), the nociceptive response to s.c. formalin injection in rats (Codene, 1993) and an NMDA-induced persistent pain model (Liu, et al., 1997).

In each of these models it is seen that the conantokin peptides, conantokin peptide derivatives and conantokin peptide chimeras have analgesic properties.

More specifically, this study evaluates the effect of intrathecal administration of conantokins in mice models of nociceptive and neuropathic pain. For nociceptive pain, the effect of the conantokins is studied in two different tests of inflammatory pain. The first is the formalin test, ideal because it produces a relatively short-lived, but reliable pain behavior that is readily quantified. There are two phases of pain behavior, the second of which is presumed to result largely from formalin-evoked inflammation of the hind paw. Conantokins are administered 10 minutes prior to injection of formalin. The number of flinches and/or the duration of licking produced by the injection is monitored. Since the first phase is presumed to be due to direct activation of primary afferents, and thus less dependent on long term changes in the spinal cord, the conantokins are presumed to have greatest effect on the magnitude of pain behavior in the second phase.

The mechanical and thermal thresholds in animals that received an injection of complete Freund's adjuvant into the hind paw are also studied. This produces a localized inflammation including swelling of the hind paw and a profound decrease in mechanical and thermal thresholds, that are detected within 24 hours after injection. The changes in thresholds in rats that receive the conantokins are compared with those of rats that receive vehicle intrathecal injections.

To evaluate the contribution of long term, NMDA receptor-mediated changes to neuropathic (i.e., nerve injury-induced) behavior, a modification of the Seltzer model of pain that has been adapted for the mouse is used. A partial transection of the sciatic nerve is first made. This also produces a significant drop in mechanical and thermal thresholds of the partially denervated hind paw. In general, the mechanical changes are more profound. They peak around 3 days after surgery and persist for months.

An important issue is whether the drugs are effective when administered after the pain model has been established, or whether they are effective only if used as a pretreatment. Clearly, the clinical need is for drugs that are effective after the pain has developed. To address this issue, animals are studied in which the conantokin is administered repeatedly, after the inflammation (CFA) or nerve injury has been established. In these experiments, the conantokins are injected daily by the intrathecal (i.t.) route. The mechanical and thermal thresholds (measured, respectively, with von Frey hairs in freely moving animals and with the Hargreave's test, also in

freely moving animals) are repeated for a 2 to 4 week period after the injury is induced and the changes in pain measured monitored over time.

It will be appreciated that the methods and compositions of the instant invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein.

- 5 It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

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- Published PCT Application WO 92/19195 (1992).
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- 45 Published PCT Application WO 95/05452 (1995).
- Published PCT Application WO 96/02286 (1996).

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Published PCT Application WO 96/40871 (1996).

5 Published PCT Application WO 96/40959 (1996).

Published PCT Application WO 97/12635 (1997).

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Cognetix, Inc.
- (ii) TITLE OF INVENTION: Use of Conantokins
- (iii) NUMBER OF SEQUENCES: 71
- (iv) CORRESPONDENCE ADDRESS:
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- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: WO
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/762,377
 - (B) FILING DATE: 06-DEC-1996
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/684,750
 - (B) FILING DATE: 22-JUL-1996
- (viii) ATTORNEY/AGENT INFORMATION:
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 - (C) REFERENCE/DOCKET NUMBER: 24260-121389-W01
- (ix) TELECOMMUNICATION INFORMATION:
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 - (B) TELEFAX: 202-962-8300

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

-61-

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Conus geographus*

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 3..14

(D) OTHER INFORMATION: /note= "Xaa at residues 3 and 4 is gamma-carboxyglutamic acid; Xaa at residues 7, 10 and 14 may be any amino acid but is preferably gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gly	Glu	Xaa	Xaa	Leu	Gln	Xaa	Asn	Gln	Xaa	Leu	Ile	Arg	Xaa	Lys	Ser
1				5				10						15	

Asn

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Conus tulipa*

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 3..13

(D) OTHER INFORMATION: /note= "Xaa at residues 3 and 4 is gamma-carboxyglutamic acid; Xaa at residues 10 and 14 may be any amino acid but is preferably gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly	Glu	Xaa	Xaa	Tyr	Gln	Lys	Met	Leu	Xaa	Asn	Leu	Arg	Xaa	Ala	Glu
1				5				10						15	

Val Lys Lys Asn Ala

20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

-62-

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Conus lynceus*

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3..15
(D) OTHER INFORMATION: /note= "Xaa at residues 3 and 4 is gamma-carboxyglutamic acid; Xaa at residues 11 and 15 may be any amino acid, but is preferably gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gly	Glu	Xaa	Xaa	Val	Ala	Lys	Met	Ala	Ala	Xaa	Leu	Ala	Arg	Xaa	Asp
1				5						10				15	

Ala Val Asn

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: *Conus radiatus*

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3..15
(D) OTHER INFORMATION: /note= "Xaa at residues 3 and 4 is gamma-carboxyglutamic acid; Xaa at residues 11 and 15 may be any amino acid, but is preferably gamma-carboxyglutamic acid"

(ix) FEATURE:
(A) NAME/KEY: Disulfide-bond
(B) LOCATION: 21..25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gly	Glu	Xaa	Xaa	Val	Ala	Lys	Met	Ala	Ala	Xaa	Leu	Ala	Arg	Xaa	Asn
1				5						10				15	

Ile	Ala	Lys	Gly	Cys	Lys	Val	Asn	Cys	Tyr	Pro
			20					25		

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Conus sulcatus*

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3..14

(D) OTHER INFORMATION: /note= "Xaa at residues 3 and 4 is gamma-carboxyglutamic acid; Xaa at residues 10 and 14 may be any amino acid, but is preferably gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly	Asp	Xaa	Xaa	Tyr	Ser	Lys	Phe	Ile	Xaa	Arg	Glu	Arg	Xaa	Ala	Gly
1				5					10					15	

Arg	Leu	Asp	Leu	Ser	Lys	Phe	Pro
							20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Conus orchroleucus*

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3..19

(D) OTHER INFORMATION: /note= "Xaa at residues 3 and 4 is gamma-carboxyglutamic acid; Xaa at residues 11 and 19 may be any amino acid, but is preferably gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gly	Glu	Xaa	Xaa	Tyr	Arg	Lys	Ala	Met	Ala	Xaa	Leu	Glu	Ala	Lys	Lys
1				5				10						15	

Ala	Gln	Xaa	Ala	Leu	Lys	Ala
						20

(2) INFORMATION FOR SEQ ID NO:7:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Conus gloriamaris*

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3..13
- (D) OTHER INFORMATION: /note= "Xaa at residue 4 is gamma-carboxyglutamic acid; Xaa at residues 9 and 13 may be any amino acid, but is preferably gamma-carboxy glutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Glu Lys Xaa Asp Arg Asn Asn Xaa Ala Val Arg Xaa Arg Leu Glu
1 5 10 15

Glu Ile

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Conus characteristicus*

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3..14
- (D) OTHER INFORMATION: /note= "Xaa at residues 3 and 4 is gamma-carboxyglutamic acid; Xaa at residues 7, 10 and 14 may be any amino acid, but is preferably gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gly Thr Xaa Xaa Asp Arg Xaa Ile Ala Xaa Tyr Val Arg Xaa Leu Glu
1 5 10 15

Glu Ala

(2) INFORMATION FOR SEQ ID NO:9:

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(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Conus quercinus*

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 3..14
 (D) OTHER INFORMATION: /note= "Xaa at residues 3 and 4 is gamma-carboxyglutamic acid; Xaa at residues 7, 10 and 14 may be any amino acid, but is preferably gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly	Tyr	Xaa	Xaa	Asp	Arg	Xaa	Val	Ala	Xaa	Thr	Val	Arg	Xaa	Leu	Asp
1				5				10						15	

Ala Ala

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys	Pro	Gly	Arg	Lys
1				5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Lys Pro Gly Arg Lys Asn
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3..4
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gly Glu Xaa Xaa
1

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3..6
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Leu Gln Xaa Asn Gln Xaa
1 5

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(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Leu Ile Arg Xaa
1

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Tyr Gln Lys Met Leu Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 4

(D) OTHER INFORMATION: /note= "Xaa is
gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Asn Leu Arg Xaa
1

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Glu Val Lys Lys Asn Ala
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 7

(D) OTHER INFORMATION: /note= "Xaa is
gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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Val Ala Lys Met Ala Ala Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note= "Xaa is
gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Leu Ala Arg Xaa
1

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Asn Ile Ala Lys Gly Cys Lys Val Asn Cys Tyr Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Asp Ala Val Asn

1

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Leu Gln Ala Asn Gln Ala

1

5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Leu Ile Arg Ala

1

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

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(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Leu Gln Ala Asn Gln Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Leu Gln Ser Asn Gln Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Leu Gln Thr Asn Gln Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3..4
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gly Asp Xaa Xaa
1

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Tyr Ser Lys Phe Ile Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:29:

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(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 4
 (D) OTHER INFORMATION: /note= "Xaa is
gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Arg Glu Arg Xaa
1

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ala Gly Arg Leu Asp Leu Ser Lys Phe Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS:
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 7

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(D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Tyr Arg Lys Ala Met Ala Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 8
(D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Leu Glu Ala Lys Lys Ala Gln Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ala Leu Lys Ala
1

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid

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- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (v) FRAGMENT TYPE: N-terminal
- (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 3..4
 - (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Gly Tyr Xaa Xaa
1
- (2) INFORMATION FOR SEQ ID NO:35:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: peptide
 - (v) FRAGMENT TYPE: internal
 - (ix) FEATURE:
 - (A) NAME/KEY: Modified-site
 - (B) LOCATION: 3..6
 - (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Asp Arg Xaa Val Ala Xaa
1 5
- (2) INFORMATION FOR SEQ ID NO:36:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: peptide
 - (v) FRAGMENT TYPE: internal

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(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Thr Val Arg Xaa
1

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Leu Asp Ala Ala
1

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3..6
- (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Asp Arg Xaa Ile Ala Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:39:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (v) FRAGMENT TYPE: C-terminal

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Leu Glu Glu Ala
1

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (v) FRAGMENT TYPE: N-terminal

- (ix) FEATURE:

- (A) NAME/KEY: Modified-site
 - (B) LOCATION: 4
 - (D) OTHER INFORMATION: /note= "Xaa is gamma-carboxyglutamic acid"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Gly Ala Lys Xaa
1

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: peptide

- (v) FRAGMENT TYPE: internal

- (ix) FEATURE:

- (A) NAME/KEY: Modified-site

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(B) LOCATION: 6

(D) OTHER INFORMATION: /note= "Xaa is
gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Asp Arg Asn Asn Ala Xaa
1 5

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 4

(D) OTHER INFORMATION: /note= "Xaa is
gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Ala Val Arg Xaa
1

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Arg Leu Glu Glu Ile
1 5

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

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(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "probe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CARGARAAYC ARGARYT

17

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 718 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Conus geographus

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 110..409

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GCGCCTTGCC TGAGGAACGA CGTGTCTTCC CCTGCCCTCT CTGTCTTCCT GACTGCAGCC	60
TTGAGCCACC CAGCCGTCAT CTCTACCATC GACTTCACCC TGATTGGCG ATG CAC	115
	Met His
	1
CTG TAC ACG TAT CTG TAT CTG CTG GTG CCC CTG GTG ACC TTC CAC CTA	163
Leu Tyr Thr Tyr Leu Tyr Leu Leu Val Pro Leu Val Thr Phe His Leu	
5 10 15	
ATC CTA GGC ACG GGC ACA CTA GAT GAT GGA GGC GCA CTG ACT GAA CGC	211
Ile Leu Gly Thr Gly Thr Leu Asp Asp Gly Gly Ala Leu Thr Glu Arg	
20 25 30	
CGT TCA GCT GAC GCC ACA GCG CTG AAA GCT GAG CCT GTC CTC CTG CAG	259
Arg Ser Ala Asp Ala Thr Ala Leu Lys Ala Glu Pro Val Leu Leu Gln	
35 40 45 50	
AAA TCC GCT GCC CGC AGC ACC GAC GAC AAT GGC AAG GAC AGG TTG ACT	307
Lys Ser Ala Ala Arg Ser Thr Asp Asp Asn Gly Lys Asp Arg Leu Thr	
55 60 65	
CAG ATG AAG AGG ATT CTC AAA CAG CGA GGA AAC AAA GCC AGA GGC GAA	355
Gln Met Lys Arg Ile Leu Lys Gln Arg Gly Asn Lys Ala Arg Gly Glu	
70 75 80	

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GAA GAA GTT CAA GAG AAT CAG GAA TTG ATC AGA GAA AAA AGT AAT GGA 403
 Glu Glu Val Gln Glu Asn Gln Glu Leu Ile Arg Glu Lys Ser Asn Gly
 85 90 95

AAA AGA TAATCAAGCT GGTGTTCCAC GTTATACCCG TCAGTTCTAA AATCCCCAGA 459
 Lys Arg
 100

TAGATCGTTC CCTATTTTTC CCACATTCTT TCTTCTCTT TTCATTTAAT TCCCCAATA 519

TTTCATGTTT ATTCTCACGT AATTGTAAAA TTTTAGGAG GAATGGTGTG TGTGTATGTG 579

CAAACGTGTAT CATAATAAA TAATGCGAAT TTAAGGAAGA AATTTTGCAG ATCCATGCAC 639

AGAAAGTCGT TAAAGACAAA TTGTATGAAT AACCAAATTT GATTGAATC AATAAAGAAC 699

CCACTAAGTG AAAAAAAAAA 718

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Met His Leu Tyr Thr Tyr Leu Tyr Leu Leu Val Pro Leu Val Thr Phe
 1 5 10 15

His Leu Ile Leu Gly Thr Gly Thr Leu Asp Asp Gly Gly Ala Leu Thr
 20 25 30

Glu Arg Arg Ser Ala Asp Ala Thr Ala Leu Lys Ala Glu Pro Val Leu
 35 40 45

Leu Gln Lys Ser Ala Ala Arg Ser Thr Asp Asp Asn Gly Lys Asp Arg
 50 55 60

Leu Thr Gln Met Lys Arg Ile Leu Lys Gln Arg Gly Asn Lys Ala Arg
 65 70 75 80

Gly Glu Glu Glu Val Gln Glu Asn Gln Glu Leu Ile Arg Glu Lys Ser
 85 90 95

Asn Gly Lys Arg
 100

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: nucleic acid

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- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "probe"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CCYTTNGCDA TRTTYTC

17

- (2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 16 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "probe"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GCCGTGCCTA GGATTA

16

- (2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 580 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Conus radiatus

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 127..447

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TTCTGTCACT TCAGATTTTCG CCGTGCCCGA GGAACGACGT GTCTTCCCTT GCTCTCTCCA 60

TCTTCCTGAC AGCAGCTTTG AGCCACCCAG CCGTCATCTC TGCCGTCGAC TTCACCCTGA 120

TTGGCG ATG CAA CTG TAC ACG TAT CTG TAT CTG CTG GTG TCC CTG GTG 168

Met Gln Leu Tyr Thr Tyr Leu Tyr Leu Val Ser Leu Val

1

5

10

[illegible]

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Met	Gln	Leu	Tyr	Thr	Tyr	Leu	Tyr	Leu	Leu	Val	Ser	Leu	Val	Thr	Phe
1				5					10					15	
Tyr	Leu	Ile	Leu	Gly	Thr	Gly	Thr	Leu	Gly	His	Gly	Gly	Ala	Leu	Thr
			20					25					30		1
Glu	Arg	Arg	Ser	Thr	Asp	Ala	Thr	Ala	Leu	Lys	Pro	Glu	Pro	Val	Leu
			35				40					45			
Leu	Gln	Lys	Ser	Ser	Ala	Arg	Ser	Thr	Asp	Asp	Asn	Gly	Asn	Asp	Arg
50						55					60				
Leu	Thr	Gln	Met	Lys	Arg	Ile	Leu	Lys	Lys	Arg	Gly	Asn	Lys	Ala	Arg
65					70					75					80

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Gly Glu Glu Glu Val Ala Lys Met Ala Ala Glu Leu Ala Arg Glu Asn
85 90 95

Ile Ala Lys Gly Cys Lys Val Asn Cys Tyr Pro
100 105

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

TGCTCGAATA AACATGAAAG ATTTGGGGAA

30

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

TCTGCGATGC AACTGTACAC GTATCTG

27

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 394 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Conus ochroleucus

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..294

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

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TAT CTG CTG GTG CCC CTG GTG ACC TTC CTC CTA ATC CTA GGC ACG GGC 48
 Tyr Leu Leu Val Pro Leu Val Thr Phe Leu Leu Ile Leu Gly Thr Gly
 1 5 10 15
 ACA CTA GAT CAT GGA GGC GCA CTG ACT GAA CGC CGT TCG ACT GAC GCC 96
 Thr Leu Asp His Gly Gly Ala Leu Thr Glu Arg Arg Ser Thr Asp Ala
 20 25 30
 ATA GCA CTG AAA CCT GAG CCT GTC CTC CTG CAG AAA TCC TCT GCC CGC 144
 Ile Ala Leu Lys Pro Glu Pro Val Leu Leu Gln Lys Ser Ser Ala Arg
 35 40 45
 AGC ACC GAC GAC AAT GGC GGC GAC AGG TTG ACT CAG ATG AAG AGG ATT 192
 Ser Thr Asp Asp Asn Gly Gly Asp Arg Leu Thr Gln Met Lys Arg Ile
 50 55 60
 CTC AAA AAG CGA GGA AAC AAA GCC AGA GGC GAA GAA GAA TAT AGA AAA 240
 Leu Lys Lys Arg Gly Asn Lys Ala Arg Gly Glu Glu Glu Tyr Arg Lys
 65 70 75 80
 GCG ATG GCA GAG CTC GAA GCT AAA AAA GCT CAA GAA GCT CTA AAG GCG 288
 Ala Met Ala Glu Leu Glu Ala Lys Lys Ala Gln Glu Ala Leu Lys Ala
 85 90 95
 GGA CGA TAATCAAGTT GGGTGTTCCTA CGTGACACTC GTCAGTTCTA AAGTCCCCAG 344
 Gly Arg
 ATAGATCGTT CCCTATTTTT GCCACATTCT TTCTTTCTCT TTTCATTAA 394

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 98 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Tyr Leu Leu Val Pro Leu Val Thr Phe Leu Leu Ile Leu Gly Thr Gly
 1 5 10 15
 Thr Leu Asp His Gly Gly Ala Leu Thr Glu Arg Arg Ser Thr Asp Ala
 20 25 30
 Ile Ala Leu Lys Pro Glu Pro Val Leu Leu Gln Lys Ser Ser Ala Arg
 35 40 45
 Ser Thr Asp Asp Asn Gly Gly Asp Arg Leu Thr Gln Met Lys Arg Ile
 50 55 60
 Leu Lys Lys Arg Gly Asn Lys Ala Arg Gly Glu Glu Glu Tyr Arg Lys
 65 70 75 80

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Ala Met Ala Glu Leu Glu Ala Lys Lys Ala Gln Glu Ala Leu Lys Ala
 85 90 95

Gly Arg

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 472 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Conus sulcatus*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 6..314

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GGGCG ATG CAA CTG TAC ACG TAT CTG TAT CTG CTG GTG CCC CTG GTG	47
Met Gln Leu Tyr Thr Tyr Leu Tyr Leu Leu Val Pro Leu Val	
1 5 10	
ACC TTC CAC CTA ATC CTA GGC ACG GGC ACA CTA GAT CAT GGA GGC GCA	95
Thr Phe His Leu Ile Leu Gly Thr Gly Thr Leu Asp His Gly Gly Ala	
15 20 25 30	
CTG ACT GAA CGC CGT TCG ACT GAC GCC ACA GCA CTG AAA CCT GAG CCT	143
Leu Thr Glu Arg Arg Ser Thr Asp Ala Thr Ala Leu Lys Pro Glu Pro	
35 40 45	
GTC CTG CAG AAA TCC GCT GCC CGC AGC ACC GAC GAC AAT GGC AAG GAC	191
Val Leu Gln Lys Ser Ala Ala Arg Ser Thr Asp Asp Asn Gly Lys Asp	
50 55 60	
AGG CTG ACT CAG ATG AAG AGG ATT CTC AAA AAG CGA GGA AAG AAT GCC	239
Arg Leu Thr Gln Met Lys Arg Ile Leu Lys Lys Arg Gly Lys Asn Ala	
65 70 75	
CGT GGC GAT GAA GAA TAT TCA AAG TTT ATA GAG AGA GAA CGC GAA GCA	287
Arg Gly Asp Glu Glu Tyr Ser Lys Phe Ile Glu Arg Glu Arg Glu Ala	
80 85 90	
GGA AGA CTG GAT TTG TCA AAA TTC CCG TGACACTCGT CAGTTCTAAA	334
Gly Arg Leu Asp Leu Ser Lys Phe Pro	
95 100	
ATCCCCAGAT AGATCGTTCC CTATTTTGT CACATTCTTT CTTTCTTTT TCATTAAATC	394

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CCCAAATCTT TCATGTTTAT TCTCACGTAA TGAATTTAAT TGTAGAATTT TTAGGGGGAA 454
GGGGGGGGGG CGAAACTG 472

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 103 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Met Gln Leu Tyr Thr Tyr Leu Tyr Leu Leu Val Pro Leu Val Thr Phe
1 5 10 15
His Leu Ile Leu Gly Thr Gly Thr Leu Asp His Gly Gly Ala Leu Thr
20 25 30
Glu Arg Arg Ser Thr Asp Ala Thr Ala Leu Lys Pro Glu Pro Val Leu
35 40 45
Gln Lys Ser Ala Ala Arg Ser Thr Asp Asp Asn Gly Lys Asp Arg Leu
50 55 60
Thr Gln Met Lys Arg Ile Leu Lys Lys Arg Gly Lys Asn Ala Arg Gly
65 70 75 80
Asp Glu Glu Tyr Ser Lys Phe Ile Glu Arg Glu Arg Glu Ala Gly Arg
85 90 95
Leu Asp Leu Ser Lys Phe Pro
100

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 379 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Conus lynceus

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..282

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

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TAT CTG CTG GTG CCC CTG GTG ACC TTC CAC CTA ATC CTA GGC ACG GGC	48
Tyr Leu Leu Val Pro Leu Val Thr Phe His Leu Ile Leu Gly Thr Gly	
1 5 10 15	
ACA CTA GAT CAT GGA GGC GCA CTG ACT GAA CGC CGT TCG ACT GAC GCC	96
Thr Leu Asp His Gly Gly Ala Leu Thr Glu Arg Arg Ser Thr Asp Ala	
20 25 30	
ATA GCA CTG AAA CCT GAG CCT GTC CTC CTG CAG AAA TCC TCT GCC CGC	144
Ile Ala Leu Lys Pro Glu Pro Val Leu Leu Gln Lys Ser Ser Ala Arg	
35 40 45	
AGC ACC GAC GAC AAT GGC AAC GAC AGG TTG ACT CAG ATG AAG AGG ATT	192
Ser Thr Asp Asp Asn Gly Asn Asp Arg Leu Thr Gln Met Lys Arg Ile	
50 55 60	
CTC AAA AAG CGA GGA AAC AAA GCC AGA GGC GAA GAG GAA GTT GCA AAA	240
Leu Lys Lys Arg Gly Asn Lys Ala Arg Gly Glu Glu Glu Val Ala Lys	
65 70 75 80	
ATG GCG GCA GAG CTT GCC AGA GAA GAC GCT GTA AAT GGG AAA	282
Met Ala Ala Glu Leu Ala Arg Glu Asp Ala Val Asn Gly Lys	
85 90	
TGATAATCAA GTTGGGTGTT CCACGTGACA CTCGTCAGTT CTAAAGTCCC CAGATAGATC	342
GTGCCCTATT TTTGCCACAT TCTTTCTTTC TCTTTT	379

(2) INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 94 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Tyr Leu Leu Val Pro Leu Val Thr Phe His Leu Ile Leu Gly Thr Gly	
1 5 10 15	
Thr Leu Asp His Gly Gly Ala Leu Thr Glu Arg Arg Ser Thr Asp Ala	
20 25 30	
Ile Ala Leu Lys Pro Glu Pro Val Leu Leu Gln Lys Ser Ser Ala Arg	
35 40 45	
Ser Thr Asp Asp Asn Gly Asn Asp Arg Leu Thr Gln Met Lys Arg Ile	
50 55 60	
Leu Lys Lys Arg Gly Asn Lys Ala Arg Gly Glu Glu Glu Val Ala Lys	
65 70 75 80	
Met Ala Ala Glu Leu Ala Arg Glu Asp Ala Val Asn Gly Lys	
85 90	

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(2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 31 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGAATTCAAT AACATGAAA GATTGGGGA A

31

(2) INFORMATION FOR SEQ ID NO:60:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 31 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGAATTCGCG ATGCAACTGT ACACGTATCT G

31

(2) INFORMATION FOR SEQ ID NO:61:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 386 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Conus gloriamaris*

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..285

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TGT CTG CTG GTG CCC CTG GTG ACC CTC TAC GTA ATT CTA GGC ACG GGC
Cys Leu Leu Val Pro Leu Val Thr Leu Tyr Val Ile Leu Gly Thr Gly
1 5 10 15

48

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ACA CTA GCT CAT GGA GGC GCA CTG ACC GAA CGC CGT TTG GCT CAC GCC	96
Thr Leu Ala His Gly Gly Ala Leu Thr Glu Arg Arg Leu Ala His Ala	
20 25 30	
AGA GCA ATG GAA CCT GAT CCT GTC CTC CTG CAG AAA TCC GCT GCC CGC	144
Arg Ala Met Glu Pro Asp Pro Val Leu Leu Gln Lys Ser Ala Ala Arg	
35 40 45	
AGC ACC GAC GAC AAC GGC AAG GAC AGG ATG ACA CAG AGG AAG AGG ATT	192
Ser Thr Asp Asp Asn Gly Lys Asp Arg Met Thr Gln Arg Lys Arg Ile	
50 55 60	
CTC AAA AAG CGA GGA AAC ACG GCC AGA GGC GCG AAA GAA GAT AGA AAT	240
Leu Lys Lys Arg Gly Asn Thr Ala Arg Gly Ala Lys Glu Asp Arg Asn	
65 70 75 80	
AAT GCG GAG GCT GTT AGA GAA AGA CTC GAA GAA ATA GGA AAA AGA	285
Asn Ala Glu Ala Val Arg Glu Arg Leu Glu Glu Ile Gly Lys Arg	
85 90 95	
TAATCAAGCT GGGTGTTC ACGTGACACTC ATCAGTTCTA AAGTCCCCAG ATAGATCGTT	345
CCCTATTTTT GCCATATTC TTTCTTTCTC TTTTCATTTA A	386

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 95 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Cys Leu Leu Val Pro Leu Val Thr Leu Tyr Val Ile Leu Gly Thr Gly	
1 5 10 15	
Thr Leu Ala His Gly Gly Ala Leu Thr Glu Arg Arg Leu Ala His Ala	
20 25 30	
Arg Ala Met Glu Pro Asp Pro Val Leu Leu Gln Lys Ser Ala Ala Arg	
35 40 45	
Ser Thr Asp Asp Asn Gly Lys Asp Arg Met Thr Gln Arg Lys Arg Ile	
50 55 60	
Leu Lys Lys Arg Gly Asn Thr Ala Arg Gly Ala Lys Glu Asp Arg Asn	
65 70 75 80	
Asn Ala Glu Ala Val Arg Glu Arg Leu Glu Glu Ile Gly Lys Arg	
85 90 95	

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

-90-

- (A) LENGTH: 356 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Conus characteristicus

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..279

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TAT CTG CTG GTG CCC CTG GTG GCC TTC CAC CTA ATC CTA GGC ACG GGC	48
Tyr Leu Leu Val Pro Leu Val Ala Phe His Leu Ile Leu Gly Thr Gly	
1 5 10 15	
ACG CTA GCT CAT GGA GAC GCA CTG ACT GAA CGC CGT TCG GCT GAT GCC	96
Thr Leu Ala His Gly Asp Ala Leu Thr Glu Arg Arg Ser Ala Asp Ala	
20 25 30	
ACA GCA CTG AAA CCT GAG CCT GTC CTC CTG CAG AAA TCC GCT GCC CGC	144
Thr Ala Leu Lys Pro Glu Pro Val Leu Leu Gln Lys Ser Ala Ala Arg	
35 40 45	
AGC ACT GAC GAC AAT GGC AAG GAC AGG TTG ACT CAG AGG AAG AGG ACT	192
Ser Thr Asp Asp Asn Gly Lys Asp Arg Leu Thr Gln Arg Lys Arg Thr	
50 55 60	
CTC AAA AAG CGA GGA AAC ATG GCC AGA GGC TAC GAA GAA GAT AGA GAG	240
Leu Lys Lys Arg Gly Asn Met Ala Arg Gly Tyr Glu Glu Asp Arg Glu	
65 70 75 80	
ATT GCG GAG ACT GTT AGA GAA CTC GAA GAA GCA GGA AAA TGAAAAAGAT	289
Ile Ala Glu Thr Val Arg Glu Leu Glu Glu Ala Gly Lys	
85 90	
AGTTCTAAAG TCCCCAGATA TATCGTTCCC TATTTTGGCC ACATTCTTTC TTTCTCTTTT	349
ATTTTAA	356

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 93 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

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Tyr Leu Leu Val Pro Leu Val Ala Phe His Leu Ile Leu Gly Thr Gly
 1 5 10 15
 Thr Leu Ala His Gly Asp Ala Leu Thr Glu Arg Arg Ser Ala Asp Ala
 20 25 30
 Thr Ala Leu Lys Pro Glu Pro Val Leu Leu Gln Lys Ser Ala Ala Arg
 35 40 45
 Ser Thr Asp Asp Asn Gly Lys Asp Arg Leu Thr Gln Arg Lys Arg Thr
 50 55 60
 Leu Lys Lys Arg Gly Asn Met Ala Arg Gly Tyr Glu Glu Asp Arg Glu
 65 70 75 80
 Ile Ala Glu Thr Val Arg Glu Leu Glu Glu Ala Gly Lys
 85 90

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 390 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Conus quercinus*

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..285

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

TAT CTG CTG GTG CCC CTG GTG GCC TTC CAC CTA ATC CTA GGC ACG GGC	48
Tyr Leu Leu Val Pro Leu Val Ala Phe His Leu Ile Leu Gly Thr Gly	
1 5 10 15	
ACG CTA GCT CAT GGA GAC GCA CGG ACT GAA CGC CGT TCG GCT GAC GCC	96
Thr Leu Ala His Gly Asp Ala Arg Thr Glu Arg Arg Ser Ala Asp Ala	
20 25 30	
ACA GCG CTG AAA CCT GAG CCT GTC CTC CTG CAG AAA TCC GCT GCC CGC	144
Thr Ala Leu Lys Pro Glu Pro Val Leu Leu Gln Lys Ser Ala Ala Arg	
35 40 45	
AGC ACT GAC GAC AAT GAC AGG GAC AGG TTG ACT CAG ATG AAG AGG ATT	192
Ser Thr Asp Asp Asn Asp Arg Asp Arg Leu Thr Gln Met Lys Arg Ile	
50 55 60	
CTC AAA AAG CGA GGA AAC ACG GCC AGA GGC TAC GAA GAA GAT AGA GAG	240
Leu Lys Lys Arg Gly Asn Thr Ala Arg Gly Tyr Glu Glu Asp Arg Glu	
65 70 75 80	

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GTT GCG GAG ACT GTC AGA GAA CTC GAC GCA GCA GGA AAA AGA AAA 285
 Val Ala Glu Thr Val Arg Glu Leu Asp Ala Ala Gly Lys Arg Lys
 85 90 95

TGATTAATCA AGCTGGGTGT TCCACTTGAC ACTCGTCAGT TCTAAAGTCA CCAGATAGAT 345

CGTTCCTGT TTTTGCCCGT TTTTCTCTT TCACTTTTCA TTAA 390

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 95 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Tyr Leu Leu Val Pro Leu Val Ala Phe His Leu Ile Leu Gly Thr Gly
 1 5 10 15

Thr Leu Ala His Gly Asp Ala Arg Thr Glu Arg Arg Ser Ala Asp Ala
 20 25 30

Thr Ala Leu Lys Pro Glu Pro Val Leu Leu Gln Lys Ser Ala Ala Arg
 35 40 45

Ser Thr Asp Asp Asn Asp Arg Asp Arg Leu Thr Gln Met Lys Arg Ile
 50 55 60

Leu Lys Lys Arg Gly Asn Thr Ala Arg Gly Tyr Glu Glu Asp Arg Glu
 65 70 75 80

Val Ala Glu Thr Val Arg Glu Leu Asp Ala Ala Gly Lys Arg Lys
 85 90 95

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /note= "Xaa is His or Gln"

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(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 12
- (D) OTHER INFORMATION: /note= "Xaa is Pro or Ser"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 15
- (D) OTHER INFORMATION: /note= "Xaa is Thr or Ala"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 16
- (D) OTHER INFORMATION: /note= "Xaa is Leu or Phe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Met Xaa Leu Tyr Thr Tyr Leu Tyr Leu Leu Val Xaa Leu Val Xaa Xaa
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Conus characteristicus

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 10..16
- (D) OTHER INFORMATION: /note= "Xaa at residues 10, 12, 13 and 16 may be any amino acid, but is preferably gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Gly Asn Asp Val Asp Arg Lys Leu Ala Xaa Leu Xaa Xaa Leu Tyr Xaa
1 5 10 15

Ile

(2) INFORMATION FOR SEQ ID NO:69

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Gly Asn Asp Val

1

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 6

(D) OTHER INFORMATION: /note= "Xaa is
gamma-carboxyglutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Asp Arg Lys Leu Ala Xaa

1

5

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Leu Tyr Glu Ile

1

WHAT IS CLAIMED IS:

1. A method for treating or preventing disorders in which the pathophysiology involves excessive excitation of nerve cells by excitatory amino acids or agonists of the NMDA receptor which comprises administering to a patient in need thereof a therapeutically effective amount of an active agent selected from the group consisting of a conantokin peptide, a conantokin peptide derivative or a conantokin peptide chimera.
5
2. The method of claim 1, wherein said disorder is a neurologic disorder or a psychiatric disorder.
10
3. The method of claim 2, wherein said neurologic disorder is a seizure.
4. The method of claim 3, wherein said seizure is seizure is associated with epilepsy.
15
5. The method of claim 2, wherein said neurologic disorder is a neurotoxic injury associated with conditions of hypoxia, anoxia or ischemia.
6. The method of claim 5, wherein said neurotoxic injury is associated with stroke, cerebrovascular accident, brain or spinal cord trauma, myocardial infarct, physical trauma, drownings, suffocation, perinatal asphyxia, or hypoglycemic events.
20
7. The method of claim 2, wherein said neurologic disorder is neurodegeneration.
8. The method of claim 7, wherein said neurodegeneration is associated with Alzheimer's disease, senile dementia, Amyotrophic Lateral Sclerosis, Multiple Sclerosis, Parkinson's disease, Huntington's disease, Down's Syndrome, Korsakoff's disease, schizophrenia, AIDS dementia, multi-infarct dementia, Binswanger dementia and neuronal damage associated with uncontrolled seizures.
25
30
9. The method of claim 2, wherein said neurologic disorder is pain.

10. The method of claim 9, wherein said pain is migraine, acute pain or persistent pain.
11. The method of claim 2, wherein said neurologic disorder is chemical toxicity.
- 5 12. The method of claim 11, wherein said chemical toxicity is addiction, morphine tolerance, opiate tolerance, opioid tolerance and barbiturate tolerance.
13. The method of claim 2, wherein said neurologic disorder is dystonia (movement
10 disorder), urinary incontinence, muscle relaxation or sleep disorder.
14. The method of claim 13, wherein said disorder is urinary incontinence.
15. The method of claim 2, wherein said psychiatric disorder is anxiety, major depression,
15 manic-depressive illness, obsessive-compulsive disorder, schizophrenia or mood disorder.
16. The method of claim 15, wherein said mood disorder is bipolar disorder, unipolar depression, dysthymia or seasonal effective disorder.
- 20 17. A method for treating memory or cognitive deficits, HIV infection, or ophthalmic indications which comprises administering to a patient in need thereof a therapeutically effective amount of an active agent selected from the group consisting of a conantokin peptide, a conantokin peptide derivative or a conantokin peptide chimera.
- 25 18. The method of claim 17 wherein said treatment is for HIV infection.
19. The method of any one of claims 1 to 18, wherein the active agent is a conantokin peptide.
- 30 20. The method of claim 19, wherein said conantokin peptide is selected from the group consisting of conantokin G having the amino acid sequence Gly-Glu-Xaa₁-Xaa₁-Leu-

Gln-Xaa₂-Asn-Gln-Xaa₂-Leu-Ile-Arg-Xaa₂-Lys-Ser-Asn (SEQ ID NO:1), conantokin T having the amino acid sequence Gly-Glu-Xaa₁-Xaa₁-Tyr-Gln-Lys-Met-Leu-Xaa₂-Asn-Leu-Arg-Xaa₂-Ala-Glu-Val-Lys-Lys-Asn-Ala (SEQ ID NO:2), conantokin R having the amino acid sequence Gly-Glu-Xaa₁-Xaa₁-Val-Ala-Lys-Met-Ala-Ala-Xaa₂-Leu-Ala-Arg-Xaa₂-Asn-Ile-Ala-Lys-Gly-Cys-Lys-Val-Asn-Cys-Tyr-Pro (SEQ ID NO:4), conatokin L having the amino acid sequence Gly-Glu-Xaa₁-Xaa₁-Val-Ala-Lys-Met-Ala-Ala-Xaa₂-Leu-Ala-Arg-Xaa₂-Asp-Ala-Val-Asn (SEQ ID NO:3), Sl having the amino acid sequence Gly-Asp-Xaa₁-Xaa₁-Tyr-Ser-Lys-Phe-Ile-Xaa₂-Arg-Glu-Arg-Xaa₂-Ala-Gly-Arg-Leu-Asp-Leu-Ser-Lys-Phe-Pro (SEQ ID NO:5), conantokin Gm having the amino acid sequence Gly-Glu-Lys-Xaa₁-Asp-Arg-Asn-Asn-Xaa₂-Ala-Val-Arg-Xaa₂-Arg-Leu-Glu-Glu-Ile (SEQ ID NO:7), conantokin Ca2 having the amino acid sequence Gly-Tyr-Xaa₁-Xaa₁-Asp-Arg-Xaa₂-Ile-Ala-Xaa₂-Tyr-Val-Arg-Xaa₂-Leu-Glu-Glu-Ala (SEQ ID NO:8), conantokin Qu having the amino acid sequence Gly-Tyr-Xaa₁-Xaa₁-Asp-Arg-Xaa₂-Val-Ala-Xaa₂-Thr-Val-Arg-Xaa₂-Leu-Asp-Ala-Ala (SEQ ID NO:9), and conantokin Cal (Con Cal) having the amino acid sequence Gly-Asn-Asp-Val-Asp-Arg-Lys-Leu-Ala-Xaa₂-Leu-Xaa₂-Xaa₂-Leu-Tyr-Xaa₂-Ile (SEQ ID NO:68), wherein Xaa₁ and Xaa₂ are γ -carboxyglutamic acid.

21. The method of any one of claims 1 to 18, wherein the active agent is a conantokin peptide derivative.
22. The method of claim 21, wherein said conantokin peptide derivative is selected from the group consisting of conantokin G having the amino acid sequence Gly-Glu-Xaa₁-Xaa₁-Leu-Gln-Xaa₂-Asn-Gln-Xaa₂-Leu-Ile-Arg-Xaa₂-Lys-Ser-Asn (SEQ ID NO:1), conantokin T having the amino acid sequence Gly-Glu-Xaa₁-Xaa₁-Tyr-Gln-Lys-Met-Leu-Xaa₂-Asn-Leu-Arg-Xaa₂-Ala-Glu-Val-Lys-Lys-Asn-Ala (SEQ ID NO:2), conantokin R having the amino acid sequence Gly-Glu-Xaa₁-Xaa₁-Val-Ala-Lys-Met-Ala-Ala-Xaa₂-Leu-Ala-Arg-Xaa₂-Asn-Ile-Ala-Lys-Gly-Cys-Lys-Val-Asn-Cys-Tyr-Pro (SEQ ID NO:4), conantokin Sl having the amino acid sequence Gly-Asp-Xaa₁-Xaa₁-Tyr-Ser-Lys-Phe-Ile-Xaa₂-Arg-Glu-Arg-Xaa₂-Ala-Gly-Arg-Leu-Asp-Leu-Ser-Lys-Phe-Pro (SEQ ID NO:5), conatokin L having the amino acid sequence Gly-Glu-Xaa₁-Xaa₁-Val-Ala-Lys-Met-Ala-Ala-Xaa₂-

-98-

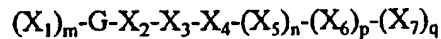
Leu-Ala-Arg-Xaa₂-Asp-Ala-Val-Asn (SEQ ID NO:3), conantokin Gm having the amino acid sequence Gly-Glu-Lys-Xaa₁-Asp-Arg-Asn-Asn-Xaa₂-Ala-Val-Arg-Xaa₂-Arg-Leu-Glu-Glu-Ile (SEQ ID NO:7), conantokin Ca2 having the amino acid sequence Gly-Tyr-Xaa₁-Xaa₁-Asp-Arg-Xaa₂-Ile-Ala-Xaa₂-Tyr-Val-Arg-Xaa₂-Leu-Glu-Glu-Ala (SEQ ID NO:8), conantokin Qu having the amino acid sequence Gly-Tyr-Xaa₁-Xaa₁-Asp-Arg-Xaa₂-Val-Ala-Xaa₂-Thr-Val-Arg-Xaa₂-Leu-Asp-Ala-Ala (SEQ ID NO:9), and conantokin Ca1 (Con Ca1) having the amino acid sequence Gly-Asn-Asp-Val-Asp-Arg-Lys-Leu-Ala-Xaa₂-Leu-Xaa₂-Xaa₂-Leu-Tyr-Xaa₂-Ile (SEQ ID NO:68), wherein Xaa₁ is γ -carboxyglutamic acid and Xaa₂ is any amino acid, with the proviso that Xaa₂ is not γ -carboxyglutamic acid.

23. The method of claim 22, wherein Xaa₂ is selected from the group consisting of Ser, Ala, Glu and Tyr.
24. The method of claim 22 or 23, wherein said derivative is further modified by deleting one or more of the amino acid residues.
25. The method of claim 22, 23 or 24, wherein said derivative is further modified by modifying one or more of the amino acid residues.
26. The method of any one of claims 1 to 18, wherein the active agent is a conantokin peptide chimera.
27. The method of claim 26, wherein said conantokin peptide chimera contains a first, second, third and fourth domain, said first domain selected from the group consisting of GE $\gamma\gamma$ (SEQ ID NO:12), GD $\gamma\gamma$ (SEQ ID NO:27), GY $\gamma\gamma$ (SEQ ID NO:34), GAK γ (SEQ ID NO:40) and GNDV (SEQ ID NO:69), said second domain selected from the group consisting of LQ γ NQ γ (SEQ ID NO:13), YQKML γ (SEQ ID NO:15), VAKMAA γ (SEQ ID NO:18), LQANQA (SEQ ID NO:22), LQANQ γ (SEQ ID NO:24), LQSNQ γ (SEQ ID NO:25), LQTNQ γ (SEQ ID NO:26), YSKF1 γ (SEQ ID NO:28), YRKAMA γ (SEQ ID NO:31), DR γ VA γ (SEQ ID NO: 35), DR γ IA γ (SEQ ID NO:38), DRNNA γ (SEQ ID

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NO:41) and DRKLA γ (SEQ ID NO: 70), said third domain selected from the group consisting of LIR γ (SEQ ID NO:14), NLR γ (SEQ ID NO:16), LAR γ (SEQ ID NO:19), LIRA (SEQ. ID NO:23), LIR γ (SEQ ID NO:14), RER γ (SEQ ID NO:29), LEAKKAQ γ (SEQ ID NO:32), TVR γ (SEQ ID NO:36), AVR γ (SEQ ID NO:42) and LE γ , and said
 5 fourth domain selected from the group consisting of KSN, AEVKKNA (SEQ ID NO:17), NIAKGCKVNCYP (SEQ ID NO:20), DAVN (SEQ ID NO:21), AGRLDLSKFP (SEQ ID NO:30), ALKA (SEQ ID NO:33), LDAA (SEQ ID NO:37), LEEA (SEQ ID NO:39), RLEEI (SEQ ID NO:43) and LYEI (SEQ ID NO:71).

- 10 28. The method of any one of claims 1 to 18, wherein the active agent is a conantokin of the generic formula



wherein

15 X_1 is Lys-Pro-Gly-Arg-Lys (SEQ ID NO:10) or Lys-Pro-Gly-Arg-Lys-Asn (SEQ ID NO:11),

X_2 is any amino acid,

X_3 is any amino acid,

X_4 is any amino acid,

X_5 is a peptide having 1-7 amino acid residues,

20 X_6 is a peptide having 1-4 amino acid residues,

X_7 is a peptide having 1-12 amino acid residues,

m , n , p , and q are independently 0 or 1, with the proviso that when m is 1, then n , p and q are each 0.

25

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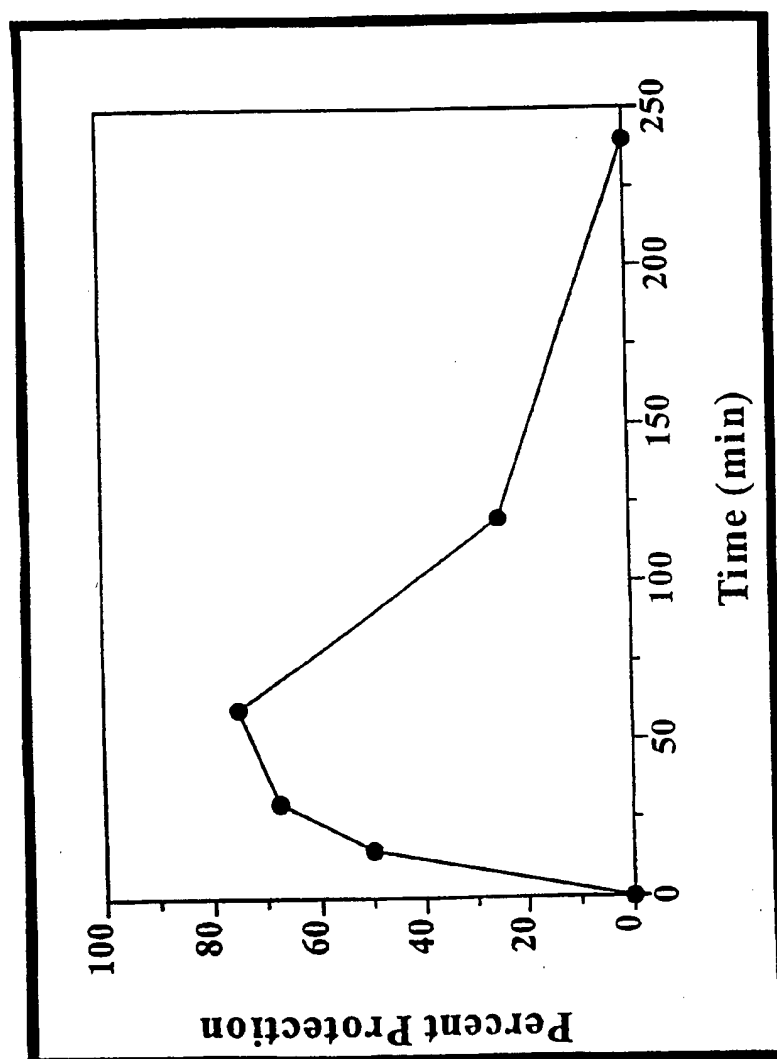


Fig. 1

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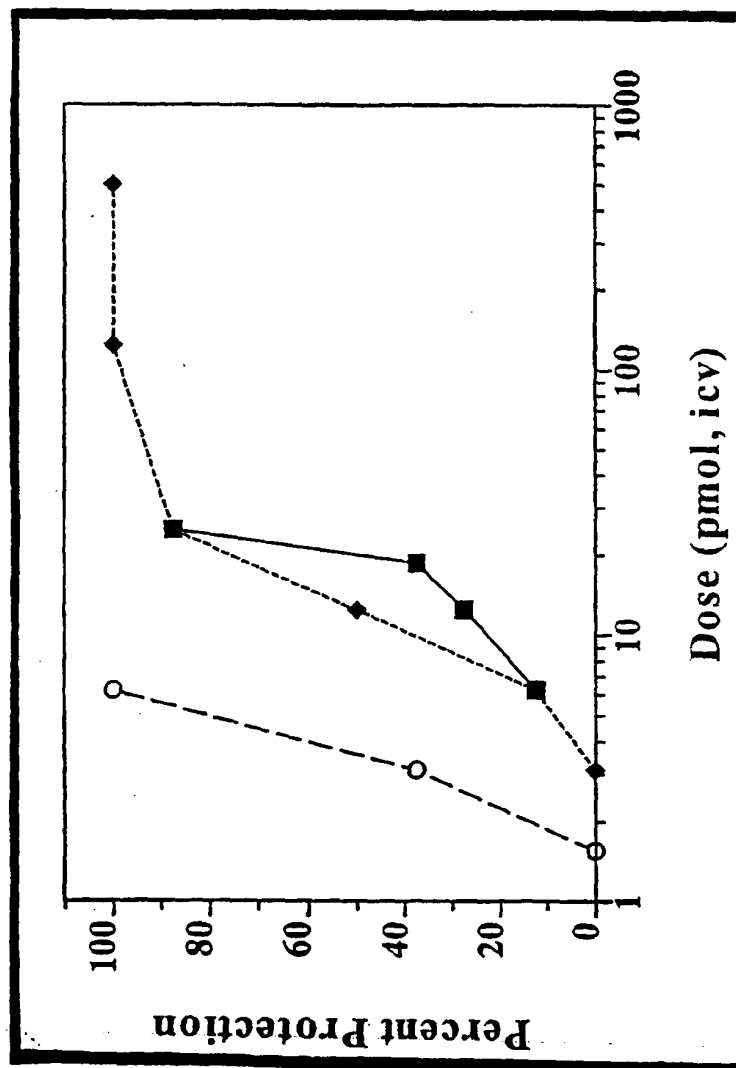


Fig. 2

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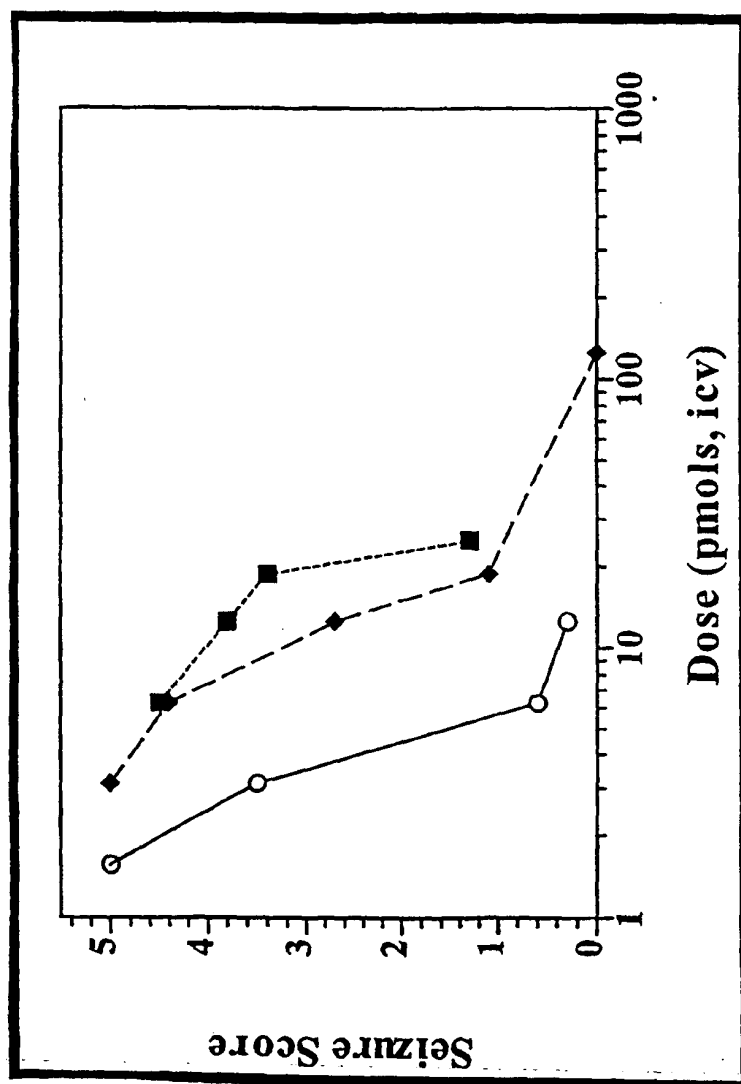


Fig. 3

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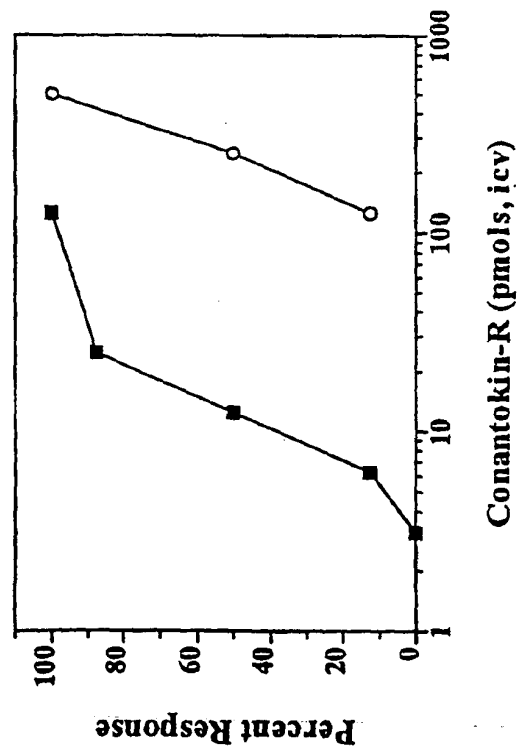


Fig. 4

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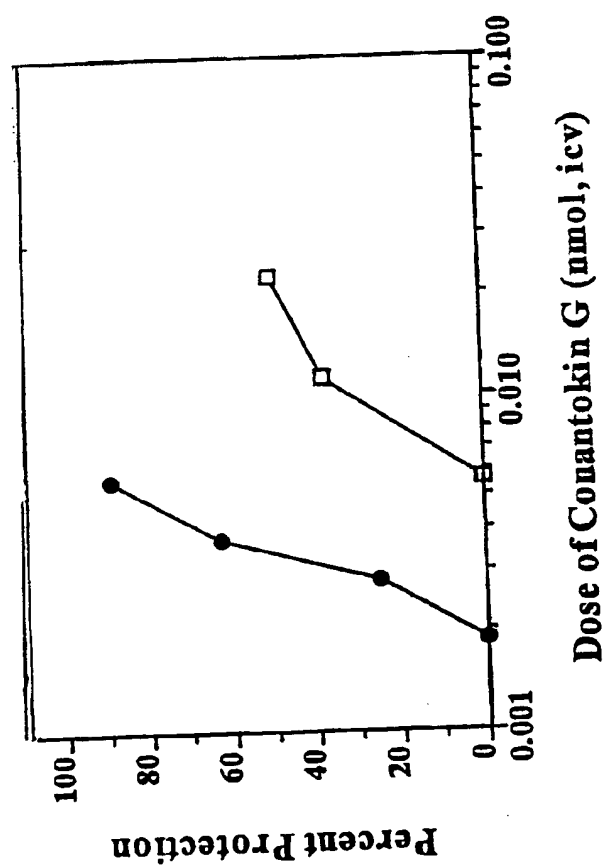


Fig. 5

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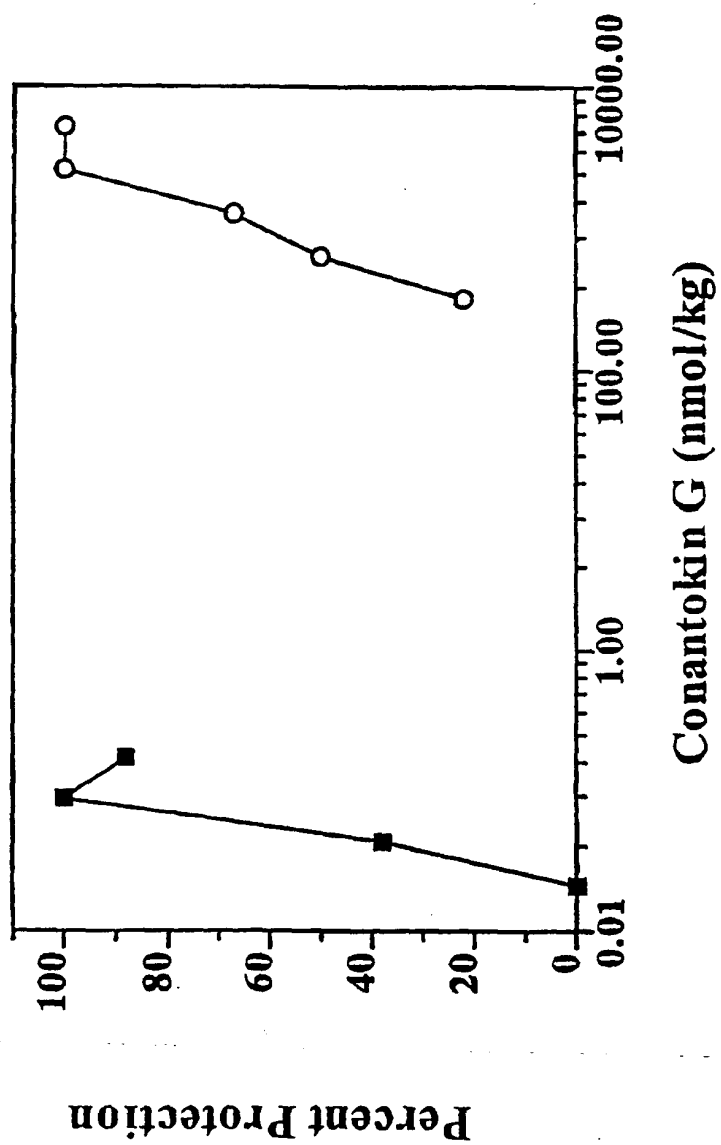


Fig. 6

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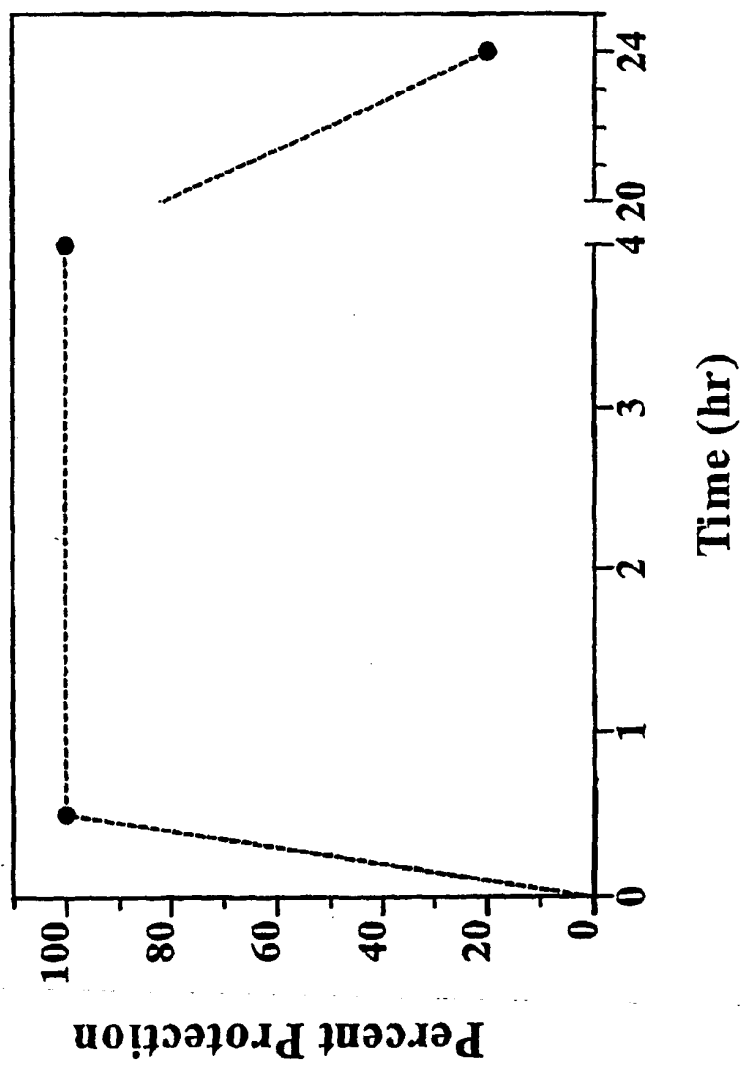


Fig. 7

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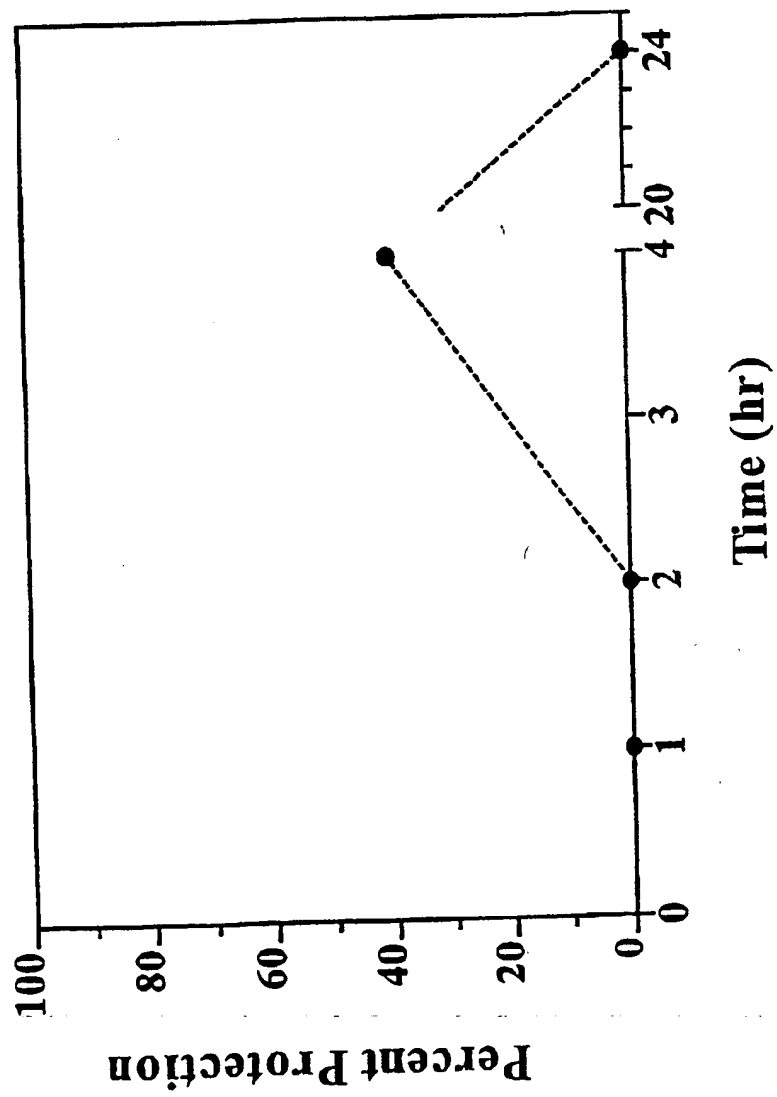


Fig. 8

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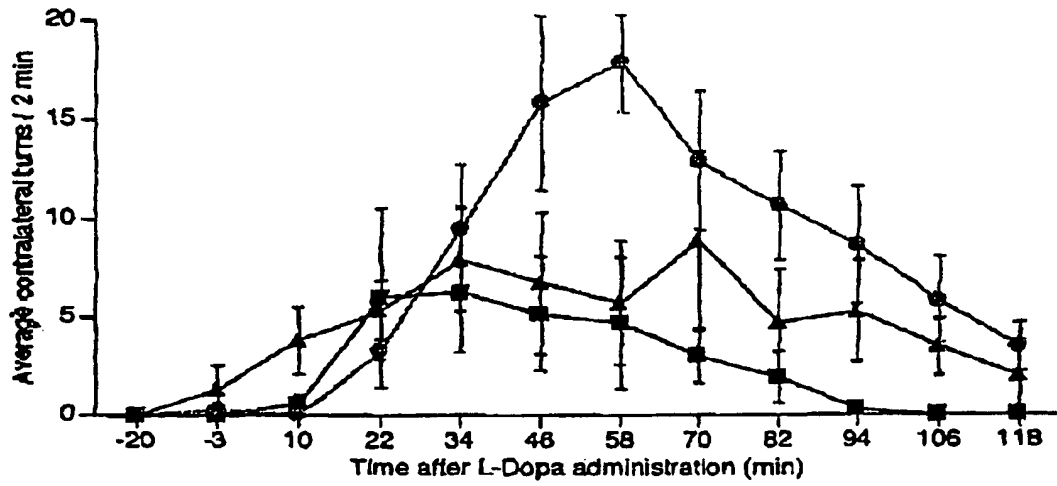


Fig. 9

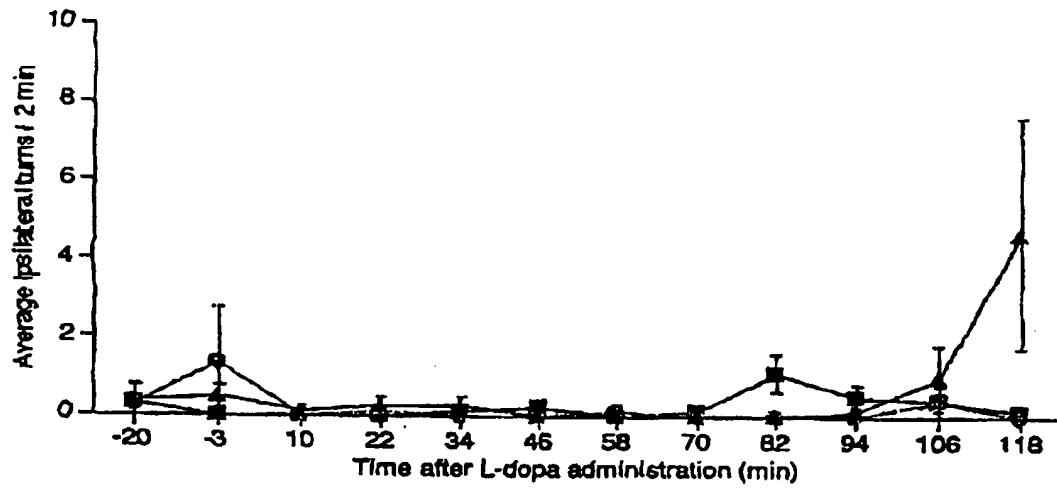


Fig. 10

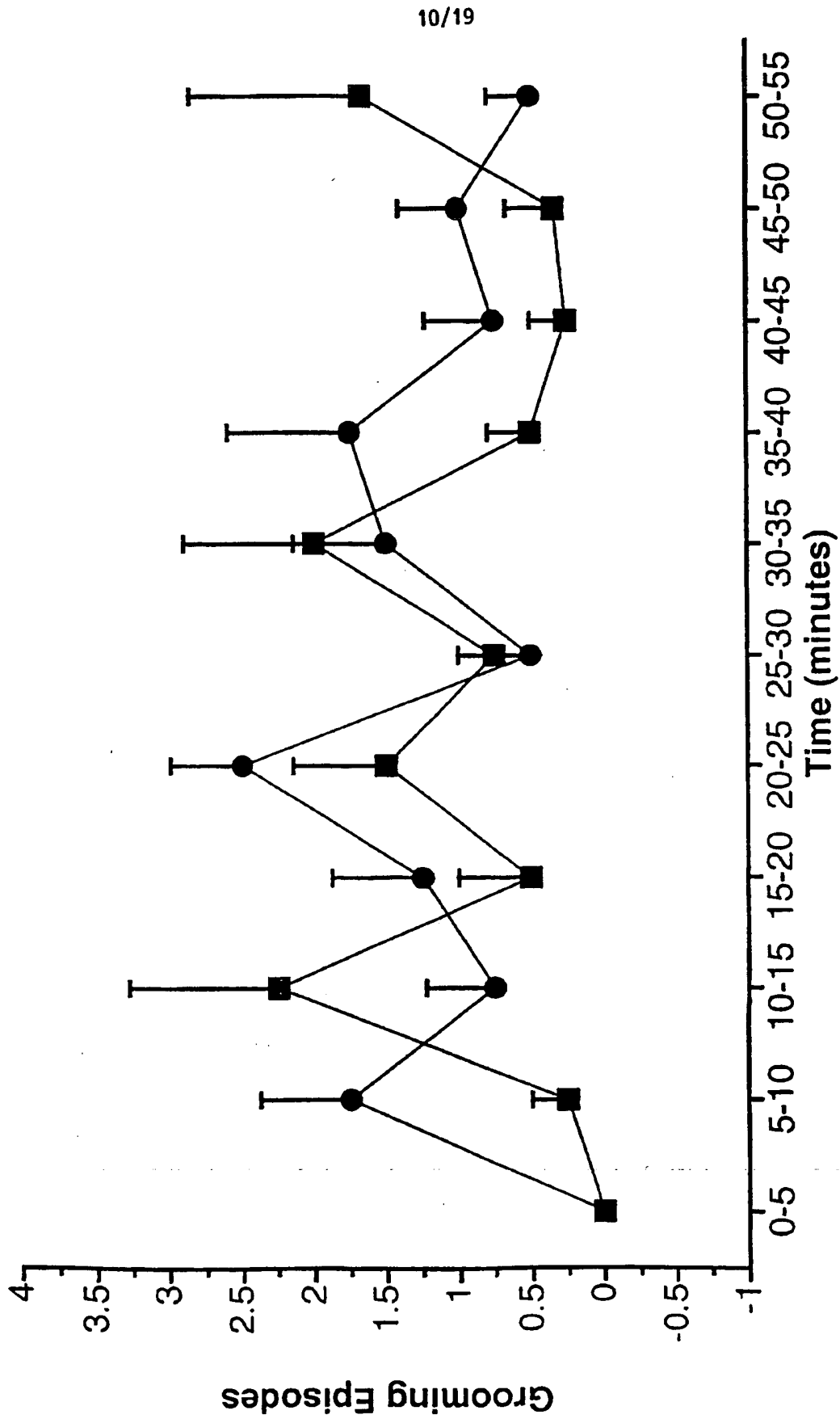


Fig. 11

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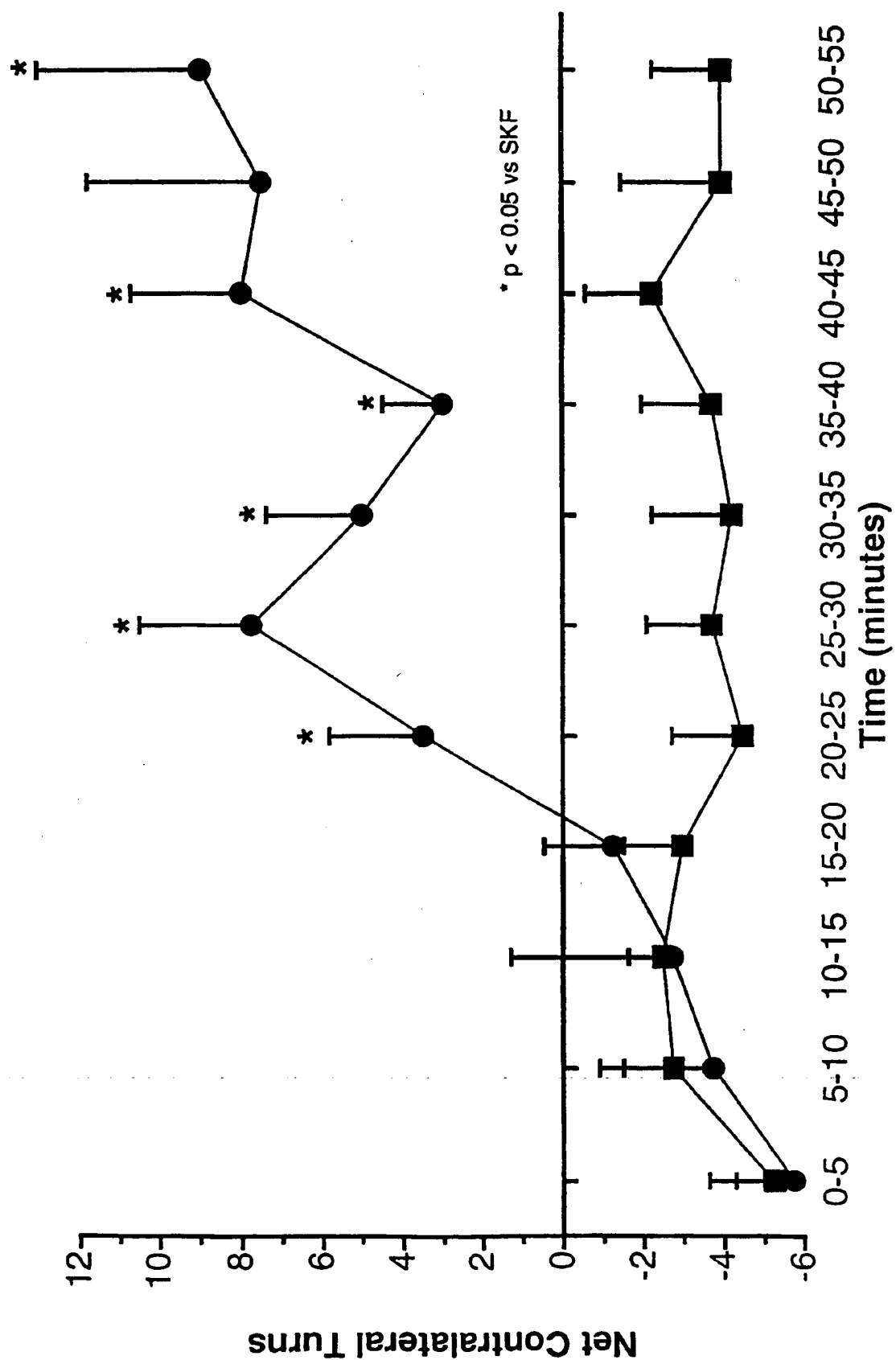


Fig. 12

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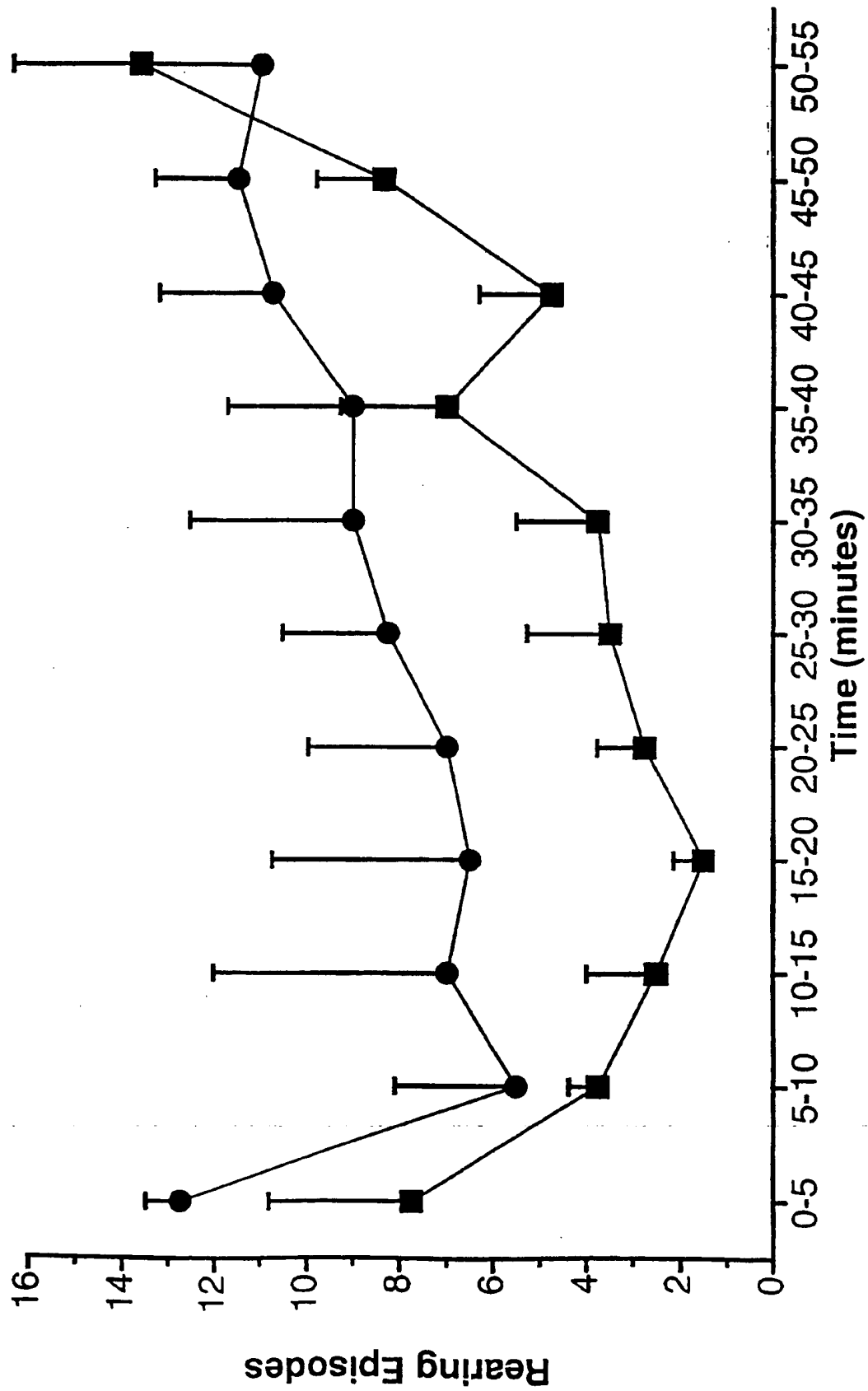


Fig. 13

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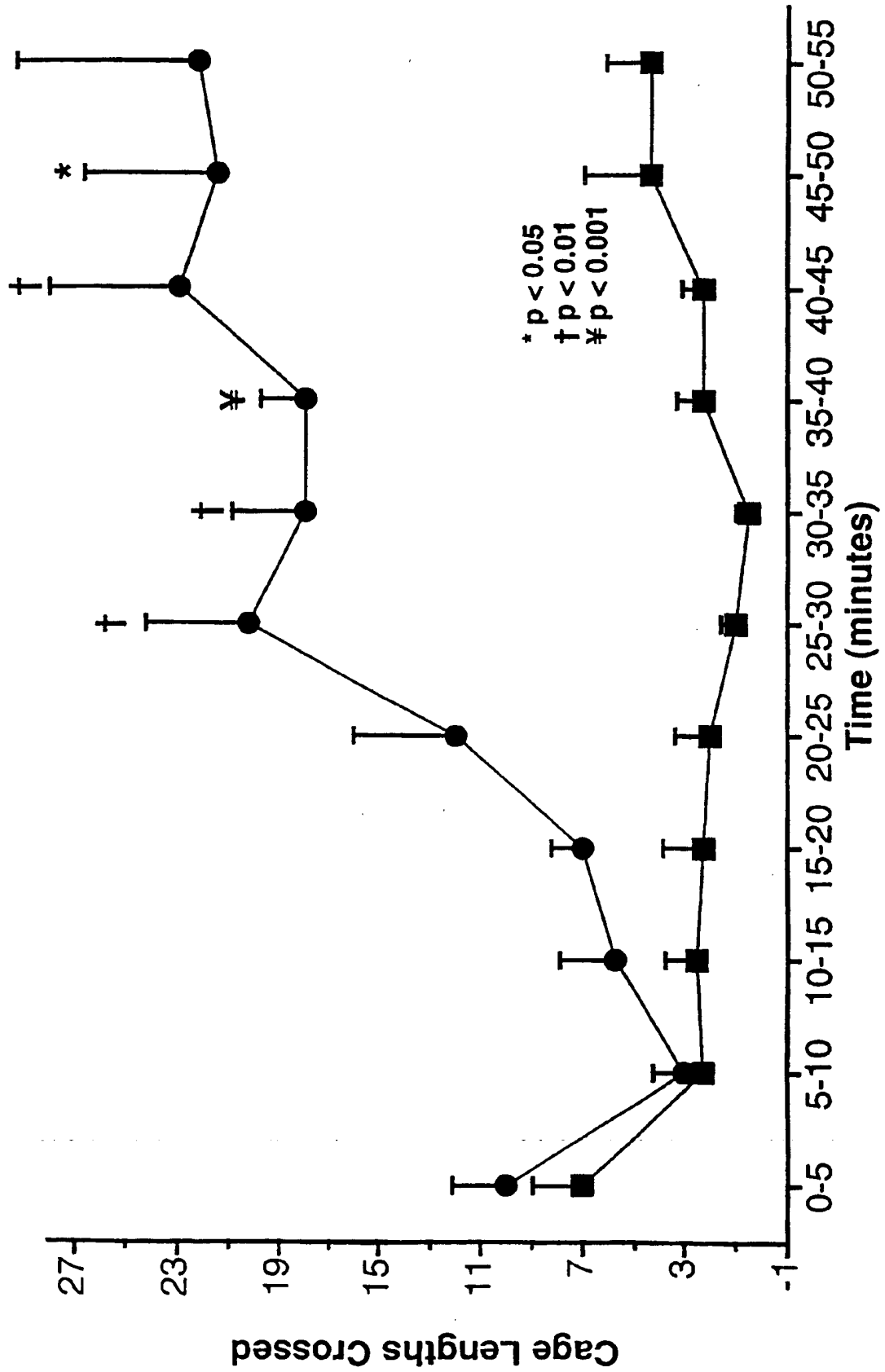


Fig. 14

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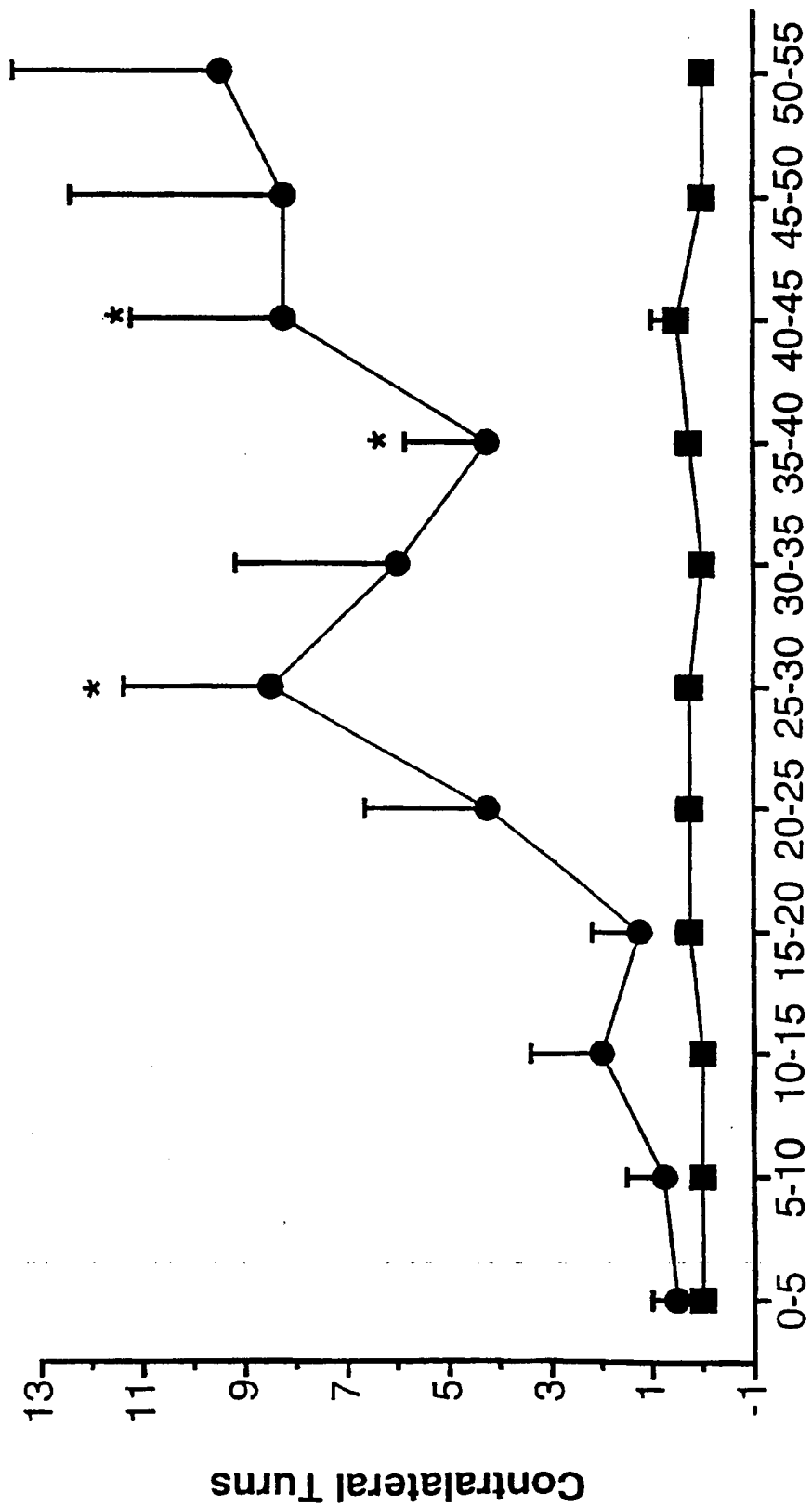


Fig. 15

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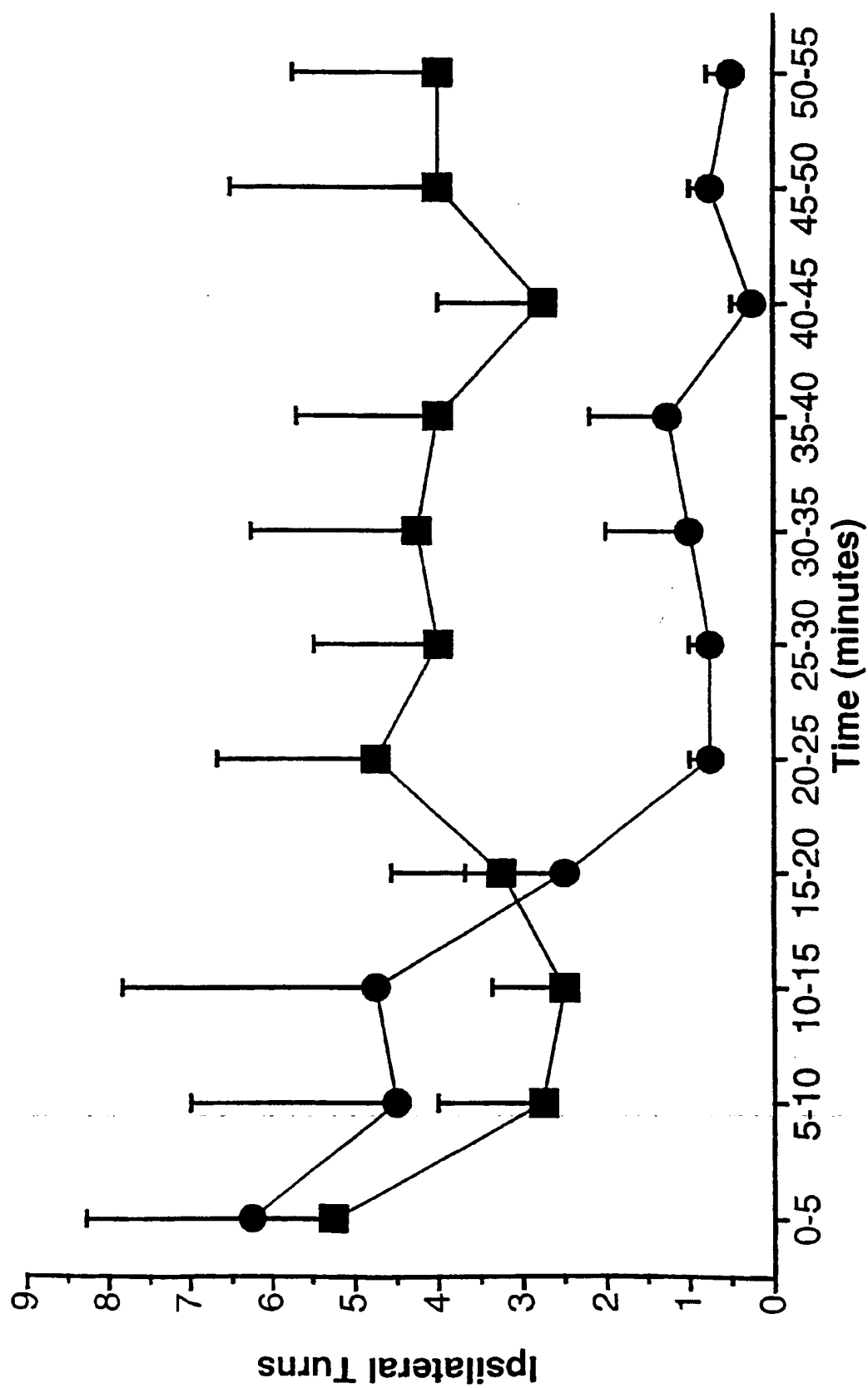


Fig. 16

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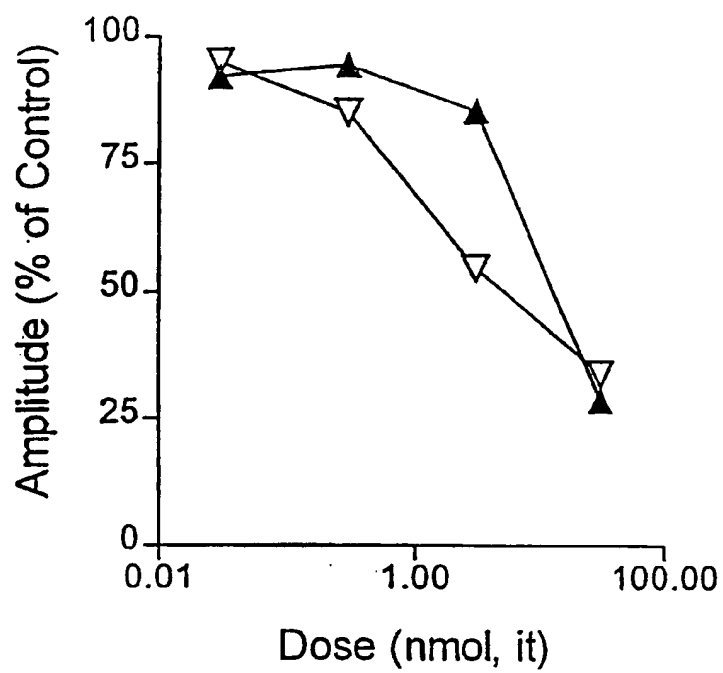


Fig. 17

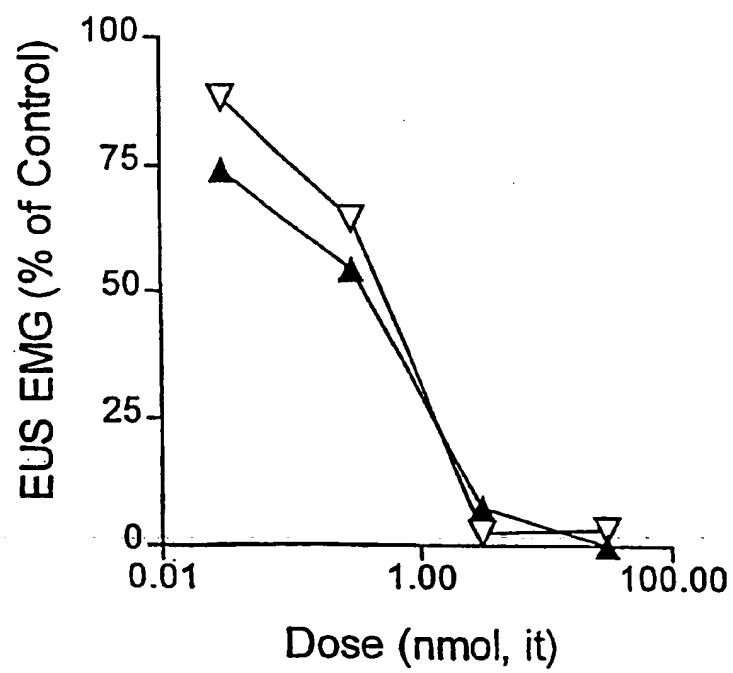


Fig. 18

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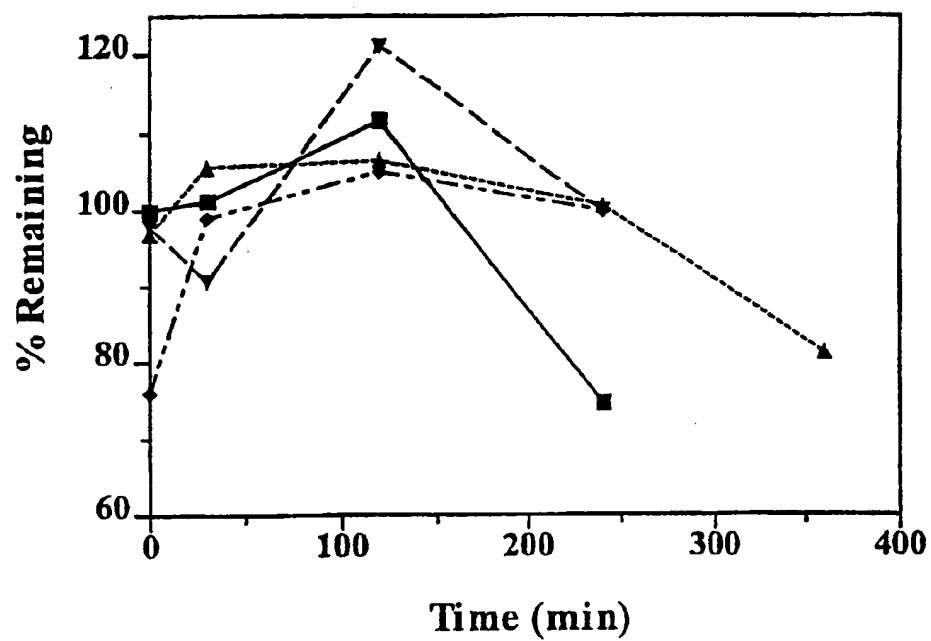


Fig. 19

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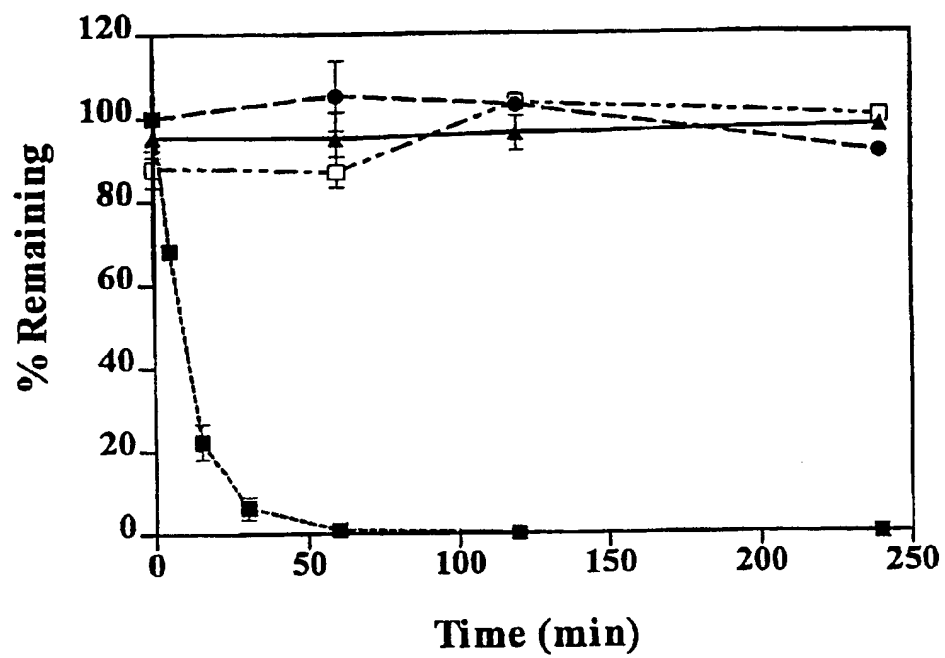


Fig. 20

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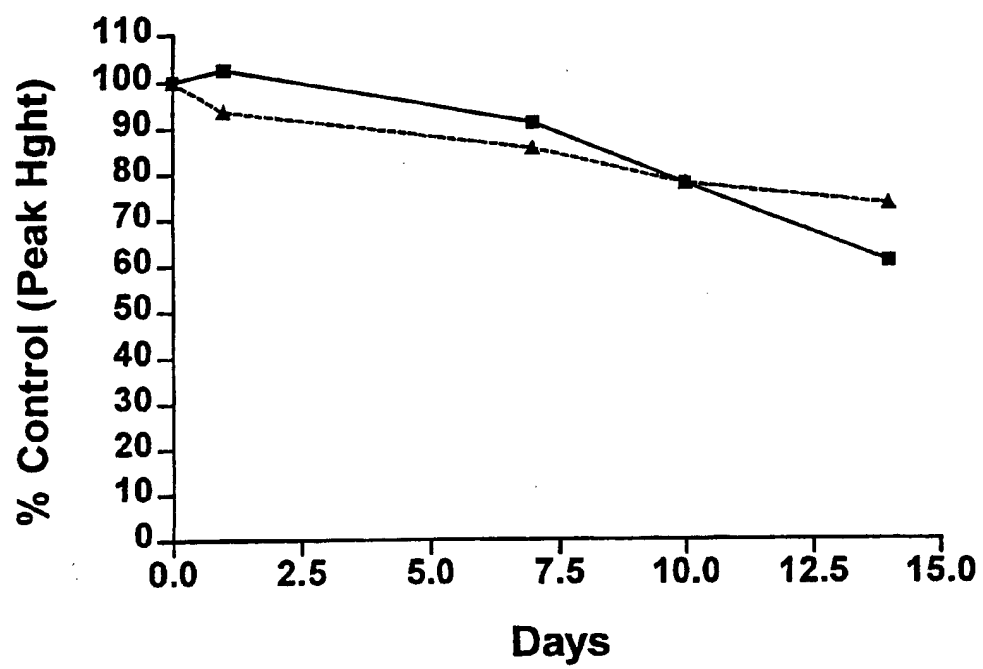


Fig. 21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/12652

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00, 38/02; C07K 5/00, 7/00.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 3, 4, 5, 6, 7, 8, 9, 10, 11; 530/324, 325, 326, 327, 328, 329, 330, 350, 300.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, CAS ONLINE.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94/07914 A1 (MACCECCHINI, MARIA-LUISA) 14 APRIL 1994 (14.04.94), see entire document.	1-24, 26-28
Y	WO 96/11698 A1 (SYMPHONY PHARMACEUTICALS, INC.) 25 APRIL 1996 (25.04.96), see entire document.	1-24, 26-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

29 OCTOBER 1997

Date of mailing of the international search report

17 NOV 1997

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/12652

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

514/2, 3, 4, 5, 6, 7, 8, 9, 10, 11; 530/324, 325, 326, 327, 328, 329, 330, 350, 300.